National Wildlife Disease Program

Fiscal Year 2011 Annual Program Report

The original artwork on this page was created by the National Wildlife Disease Program's Erika Kampe and Sarah Goff

USDA United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

National Wildlife Disease Program

FY 2011 Annual Program Report

Staff

National Wildlife Disease Coordinator
Thomas DeLiberto

Program Managers
Dale Nolte
Thomas Gidlewski

Budget Analyst
Mary Kimball

Wildife Disease Biologists
John Baroch
Sarah Bevins
Dennis Kohler
Mark Lutman
Kerri Pedersen
Brandon Schmit

Biological Science Technician
Emily Blizzard

Agency/Organization Acronym List

APHIS - Animal Plant Health Inspection Service
ASU - Arizona State University
ARS - Agricultural Research Service
CDC - Centers for Disease Control
CSU - Colorado State University
FDA - Food and Drug Administration
FADDL - Foreign Animal Disease Diagnostic Laboratory
NAHLN - National Animal Health Laboratory Network
NIH - National Institutes of Health
NVSL - National Veterinary Services Laboratories
NWDP - National Wildlife Disease Program
NWHC - National Wildlife Health Center
NWRC - National Wildlife Research Center
SCWDS - Southeastern Cooperative Wildlife Disease Study
SEPRL - Southeast Poultry Research Laboratory
USDA - United States Department of Agriculture
USFWS- United State Fish and Wildlife Service
VS - Veterinary Services
WS - Wildlife Services

Species List

Species List

***Alces alces*: Moose**
***Anas discors:* Blue-winged teal**
***Anas platyrhynchos*: Mallard Duck**
***Anser albifrons:* White-fronted geese**
***Anser indicus*: Bar-headed Goose**
***Bison bison*: Bison**
***Bos primigenius:* Cattle**
***Branta canadensis*: Canada Goose**
***Canis latrans*: Coyote**
***Canis lupus dingo*: Dingos**
***Canis lupus familiaris*: Domestic dog**
***Canis lupus*: Wolves**
***Capra aegagrus hircus*: Goats**
***Cervus canadensis*: Elk**
***Chen caerulescens caerulescens*: Lesser Snow Goose**
***Chrysops* spp.: Deer flies and others**
***Crocidura leucodon*: White-toothed Shrew**
***Cygnus olor*: Mute Swan**
***Cynomys* spp.: Prairie Dogs**
***Dermacentor andersoni* : Rocky Mountain Wood Tick**
***Didelphis virginiana:* Opossums**
***Equus ferus caballus*: Horses**
***Felis catus*: Domestic cats**
***Gallus gallus domesticus*: Chickens**
***Lama glama*: Llamas**
***Larus glaucoides:* Iceland gulls**
***Lynx canadensis*: Lynx**
***Lynx rufus*: Bobcat**
***Odocoileus hemionus*: Mule deer**
***Odocoileus virginianus*: White-tailed deer**
***Ovis aries*: Sheep**
***Procyon lotor*: Raccoon**
***Sus scrofa:* Feral Swine**
***Urocyon cinereoargenteus*: Grey fox**
***Ursus americanus*: Black bear**
***Vulpes vulpes*: Red fox**

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NWDP Surveillance Projects

Classical Swine Fever Negative Cohort

Feral swine are considered an invasive exotic species within the United States. Their populations continue to expand and, currently, an estimated 5 million individuals occupy at least 38 states. Feral swine are competent hosts for a number of foreign animal diseases that pose a very real threat to the health and economic viability of the United States livestock industry. Although diseases such as classical swine fever (CSF), Foot-and-Mouth Disease (FMD), and African swine fever (ASF) are not currently found within the United States, feral swine have been identified as a high risk pathway for the transmission of foreign animal diseases into susceptible wildlife and domestic livestock populations. Surveillance for foreign animal diseases in wildlife requires a robust and proactive approach because the majority of individuals within populations of interest ultimately remain unobserved and untested.

Classical swine fever is a highly contagious viral septicemia affecting only swine. Also known as hog cholera, it has been successfully eradicated from many developed nations with extensive swine production, but is still endemic in much of the world. Transmission is typically the result of direct or indirect contact with infectious bodily fluids or consumption of contaminated tissues. Outbreaks in countries free of CSF can have a severe impact on producers due to high swine mortality, the curtailment on exportation of swine and pork products, and from costs incurred to control and eradicate the disease.

Table 1: Number of feral swine tested for Classical Swine Fever, Fiscal Year 2006-2011.

FY2006	FY2007	FY2008	FY2009	FY2010	FY2011
21	1138	2098	2111	2560	3156

Figure 1: Feral Swine

The NWDP conducts surveillance in feral swine across the Nation. This effort, combined with other surveillance streams in domestic animals and pork products, ensure early detection of CSF if introduced. It also minimizes the risk of transmission to the domestic swine industry, as well as demonstrate freedom from disease for trading partners.

The CSF surveillance program was initiated in October 2005 and efforts are aligned with the Federal fiscal year, which begins 1 October and continues through 30 September. Nationally, there is variability in feral swine densities, distributions, WS and state/local/ tribal agency control infrastructure, and laws limiting capture methods. As a result, surveillance methodology varies accordingly. The NWDP usually samples feral swine that are taken for other wildlife damage

NWDP Surveillance Projects

Classical Swine Fever Negative Cohort, continued

management purposes by APHIS-WS. In some instances, however, the NWDP and other WS personnel will proactively capture and test feral swine for disease. Trapping, snaring, and aerial gunning are the primary methods used to remove feral swine. Blood is collected post-mortem from each pig, centrifuged, and the serum is aliquoted into cryogenic vials that are sent to the APHIS/VS FADDL on Plum Island, New York for CSF antibody testing. When blood is not available, tonsils are submitted to the NAHLN for CSF testing by real-time polymerase chain reaction. The majority of samples are taken from feral swine populations considered to be at high risk of a foreign animal disease introduction like CSF.

The total numbers of samples that have been collected each fiscal year from the inception of the surveillance program are listed in Table 1. In Fiscal Year 2011, 12 tonsil and 3,144 serum samples were collected in 32 states; all were negative for CSF. The numbers of samples collected specifically for CSF testing by state during Fiscal Year 2011 are listed in Table 2.

Table 2: Feral swine tested for Classical Swine Fever, by state, during Fiscal Year 2011

State	# of samples collected for CSF testing
Alabama	100
Arkansas	193
Arizona	31
California	134
Florida	379
Georgia	204
Hawaii	221
Iowa	1
Illinois	16
Indiana	17
Kansas	92
Kentucky	40
Louisiana	178
Michigan	16
Missouri	207
Mississippi	162
North Carolina	89
New Hampshire	15
New Jersey	2
New Mexico	73
Nevada	9
New York	6
Ohio	3
Oklahoma	263
Oregon	47
Pennsylvania	2
South Carolina	58
Tennessee	37
Texas	533
Virginia	22
Wisconsin	5
West Virginia	1

NWDP Surveillance Projects

Pseudorabies

Pseudorabies virus (PRV), also known as Aujeszky's disease, is a viral disease in the family *Herpesviridae* that is an economically important disease in domestic swine. Although it is endemic in most parts of the world, it has been eradicated from commercial swine in the United States. However, feral swine are known reservoirs of the disease and could potentially serve as a proximate or ultimate source for reintroduction into commercial swine. Feral swine populations often overlap with domestic swine operations, which could lead to disease transmission opportunities.

Transmission of PRV occurs primarily through direct animal-to-animal contact. In feral swine, there are no clinical signs of the disease or mortality. Other domestic and wild mammals such as cattle, horses, sheep, goats, dogs, and raccoons can be susceptible, and the disease is often fatal in these species. Humans are not susceptible to PRV.

Table 1: Number of feral swine samples screened for pseudorabies, Fiscal Year 2006-2011.

FY2007	FY2008	FY2009	FY2010	FY2011
1255	2564	2449	2563	3161

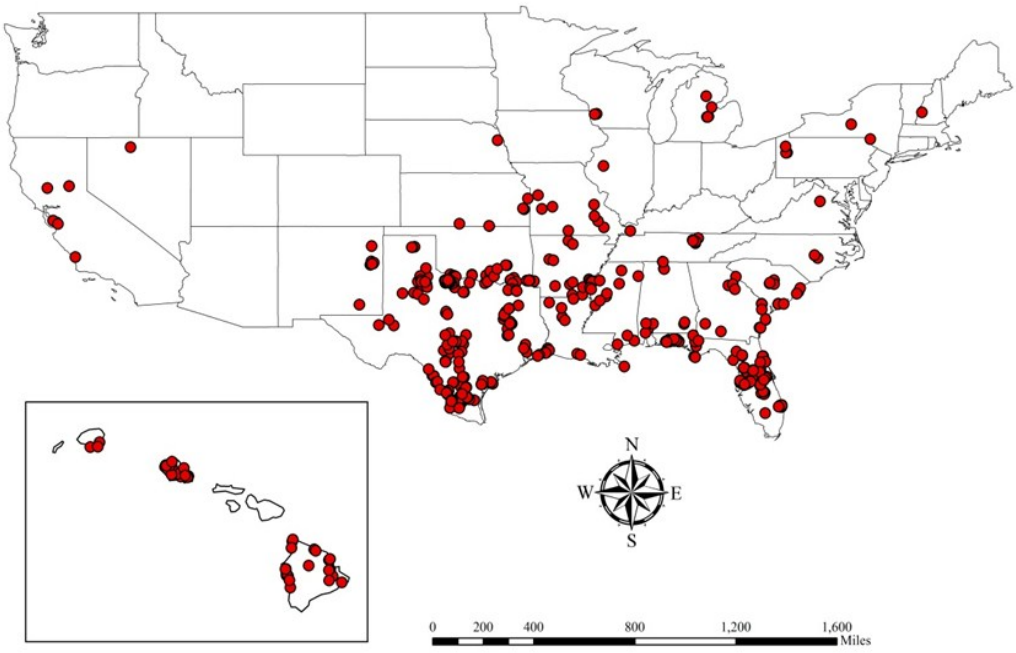


Figure 1: Pseudorabies positive sampling locations from Fiscal Year 2006-2011.

Feral swine are considered an invasive species in the United States and are estimated to cause millions of dollars in damage each year. They are currently known to exist in 38 states with a population estimate of 5 million, but each year they expand into new territory. Wildlife Services' personnel remove approximately 30,000 feral swine each year for wildlife damage management purposes. The NWDP takes advantage of these removal activities to collect samples for disease surveillance.

The objective for monitoring PRV in feral swine is to establish baseline data and identify trends of

NWDP Surveillance Projects

Pseudorabies, continued

prevalence. This data can be used to ensure appropriate levels of biosecurity are implemented on farms in an area, relative to the level of PRV circulating in the local feral swine population.

Trapping and aerial hunting are the primary methods used to remove feral swine. Samples are collected from these animals whenever possible, especially in new counties where feral swine have not previously been sampled. Blood is collected from each pig, centrifuged, and the serum is aliquoted into cryogenic vials. Samples are shipped to the NWDP Feral Swine Tissue Archive in Fort Collins, Colorado. When sufficient samples have been collected, they are batched and shipped to the Washington Animal Disease Diagnostic Laboratory or the Wisconsin Veterinary Diagnostic Laboratory for testing using a gB enzyme-linked immunosorbent assay.

The feral swine sampling program was initiated in October 2006. The total number of samples that have been collected each fiscal year are listed in Table 1. The number of samples collected for PRV testing by state during Fiscal Year 2011 is listed in Table 2 as well as the number of positive samples collected during the same year. Figure 1 depicts the sites where PRV positive pigs have been collected since the initiation of surveillance in late 2006.

Table 2: Number of pseudorabies samples collected, by state, in Fiscal Year 2011.

State	# of samples collected for PRV testing	# of positive samples
Alabama	100	14
Arkansas	186	21
Arizona	31	0
California	134	7
Florida	376	151
Georgia	204	12
Hawaii	221	91
Iowa	1	0
Illinois	16	1
Kansas	92	12
Kentucky	35	1
Louisiana	179	27
Michigan	14	1
Missouri	198	12
Mississippi	210	23
North Carolina	88	4
New Hampshire	15	1
New Jersey	2	0
New Mexico	73	4
Nevada	9	1
New York	6	0
Ohio	3	0
Oklahoma	264	39
Oregon	47	0
Pennsylvania	2	0
South Carolina	58	8
Tennessee	36	4
Texas	531	137
Virginia	22	2
Wisconsin	7	1
West Virginia	1	0

NWDP Surveillance Projects

Swine Brucellosis

Feral swine are considered an invasive exotic species within the United States. They carry a number of endemic diseases that can pose a risk to humans, as well as to cattle and domestic swine. One such disease is swine brucellosis, caused by the bacterium *Brucella suis*. There are several recognized species of *Brucella*, and each is associated with a specific animal host. While *B. suis* primarily infects pigs, it also can cause disease in cattle, horses, dogs, and humans. Similarly, swine also may become infected with *B. abortus* or *B. melitensis*. The primary route of transmission for *B. suis* in feral swine is thought to be venereal, but vertical transmission via infected milk or oral exposure to infected tissues, such as aborted fetuses, and placental tissues also has been documented.

The commercial swine industry in the United States maintains brucellosis-free status in all states, but the presence of brucellosis infected feral swine populations and the potential for them to transmit disease to domestic swine could jeopardize the commercial swine industry. Improved understanding of the prevalence and geographic distribution of brucellosis in feral swine is important for informing and guiding relevant management decisions that will help ensure the security of the United States swine and cattle industries. In addition, feral swine are known to carry other zoonotic *Brucella* species. Brucellosis in humans can manifest as recurrent fever, chills, headaches, and general weakness, and can afflict those infected for extended periods of time. Hunters, wildlife biologists, and anyone involved in butchering or dressing infected feral swine are at risk.

Figure 1: Feral swine

Surveillance is conducted to improve our understanding of the apparent prevalence and geographic distribution of brucellosis in feral swine. Such knowledge allows us to increase our ability to identify areas of increased risk for reintroduction of brucellosis into domestic swine and cattle populations, as well as areas of higher risk for hunters and others who have contact with feral swine.

Trapping, snaring, and aerial gunning are the primary methods used to remove feral swine. Blood is collected post-mortem from each pig, centrifuged, and the serum is aliquoted into cryogenic vials that are shipped to the NWDP in Fort Collins, Colorado for screening using the brucellosis card test. Presumptive positive samples are then batch shipped for confirmatory testing using fluorescence polarization assay (FPA) at the Kansas State-Federal Brucellosis Laboratory.

The total numbers of samples that have been collected each fiscal year from the inception of the surveillance program are listed in Table 1. The numbers of samples collected specifically for swine brucellosis testing by state during Fiscal Year 2011 are listed in Table 2.

Table 1: Number of samples screened for swine brucellosis, Fiscal Years 2006-2011.

FY2006	FY2007	FY2008	FY2009	FY2010	FY2011
2	1240	2474	2730	2540	3150

NWDP Surveillance Projects

Swine Brucellosis, continued

Table 2: Number of samples screened for swine brucellosis by state, and number of positive samples, in Fiscal Year 2011.

State	# of samples collected for swine brucellosis testing	# of positive samples
Alabama	100	6
Arkansas	184	4
Arizona	31	0
California	134	1
Florida	378	27
Georgia	204	6
Hawaii	220	22
Iowa	1	0
Illinois	16	0
Kansas	90	0
Kentucky	35	0
Louisiana	179	3
Michigan	14	0
Missouri	197	1
Mississippi	210	1
North Carolina	85	5
New Hampshire	15	0
New Jersey	2	0
New Mexico	72	0
Nevada	9	0
New York	6	0
Ohio	3	0
Oklahoma	264	22
Oregon	47	0
Pennsylvania	2	0
South Carolina	58	11
Tennessee	36	0
Texas	528	13
Virginia	22	0
Wisconsin	7	0
West Virginia	1	0

NWDP Surveillance Projects

Feral Swine Negative Cohort Study

The NWDP provided feral swine samples as part of a larger negative cohort project looking at African swine fever (ASF) and Foot-and-Mouth Disease (FMD) diagnostic and communication protocols. Collaborators included the National Animal Health Laboratory Network (NAHLN) and USDA/APHIS VS. Foot-and-Mouth Disease and ASF are classified as foreign animal diseases in the United States. Foreign animal disease surveillance programs targeting susceptible livestock and wildlife populations are becoming increasingly important as globalized trade and increased movements of people create additional opportunities for introductions of potentially harmful pathogens into the United States.

Foot-and-Mouth Disease exists in roughly two thirds of the world and outbreaks continue to create large economic impacts in countries that have been burdened with the disease. African swine fever is a contagious, often fatal disease of swine that is clinically indistinguishable from classical swine fever and has caused recent outbreaks in Italy, Russia, and the Republic of Georgia. Foot-and-Mouth Disease and ASF do not cause disease in humans, but their risk to the United States swine industry warrants validation of testing protocols to support future surveillance programs incorporating these diseases.

The main objective of the project was to provide negative samples that were used to validate the real-time reverse-transcription polymerase chain reaction tests for FMD and ASF in feral swine. Secondary objectives included building field personnel experience and laboratory capacity regarding FMD/ASF surveillance in feral swine, and assessing and improving laboratory procedures and processes related to

Figure 1: Feral swine in Florida.

NWDP Surveillance Projects

Negative Cohort Study, continued

FMD/ASF sample selection, testing, and communication of results.

Trapping, snaring, and aerial gunning were the primary methods used by the NWDP wildlife disease biologists and WS personnel to obtain feral swine for sampling. Oral swabs were collected post-mortem from each pig and placed into vials containing Dulbecco's Modified Eagle's Medium for FMD analysis whereas whole blood samples in EDTA tubes were submitted for ASF testing. All samples were sent to select NAHLN laboratories for testing. The majority of samples were taken from feral swine in areas identified as high risk for FMD or ASF introduction.

The NWDP collected feral swine samples from July 2010 through March 2011. In total, 1,223 samples were collected for the study comprising 768 oral swabs in 11 states for FMD test validation and 455 whole

Table 1: Total number of Foot-and-Mouth Disease, and African Swine Fever, negative cohort samples collected by state.

State	Number of FMD Samples	Number of ASF Samples
Arkansas	47	0
California	57	68
Florida	73	64
Georgia	132	58
Hawaii	105	76
Kansas	33	32
Kentucky	0	11
Mississippi	42	47
North Carolina	64	0
Oklahoma	49	99
South Carolina	13	0
Texas	153	0
TOTAL	768	455

NWDP Surveillance Projects

Swine Influenza

Swine play a unique role in the epidemiology of influenza A viruses. They have similar cellular receptors to both birds and humans and, consequently, can become infected with subtypes of influenza A viruses associated with people and birds. If different influenza A subtypes are present within an individual, there is an opportunity for genetic re-assortment to occur between subtypes. This sort of mutation, known as antigenic shift, causes a sudden and significant change in the genetic make-up of a virus, which may lead to the creation of a potentially more infectious subtype. Influenza virus in swine has received increasing attention since the emergence of the novel pH1N1 subtype (an influenza subtype that affects humans, domestic swine and several other species) in March, 2009. H1N1 was the dominant influenza subtype in the United States domestic swine populations since its discovery in the early 1930s; however, a new subtype (H3N2) created by a triple re-assortment of avian, human, and porcine genes was discovered in 1998 in commercial swine populations. This is now the dominant subtype across the country. The 2009 novel pH1N1 is similar to H3N2 in that it contains an assortment of genes from humans, birds, and pigs. This subtype also contains swine genes of Eurasian origin that had not previously been detected in North American swine.

Feral swine pose a risk of introducing or reintroducing endemic and foreign animal diseases to American livestock and wildlife populations. Feral swine can also serve as a reservoir for zoonotic diseases that are a great concern to human health. Wildlife Services is working with several federal agencies to establish surveillance programs that will detect influenza viruses, which

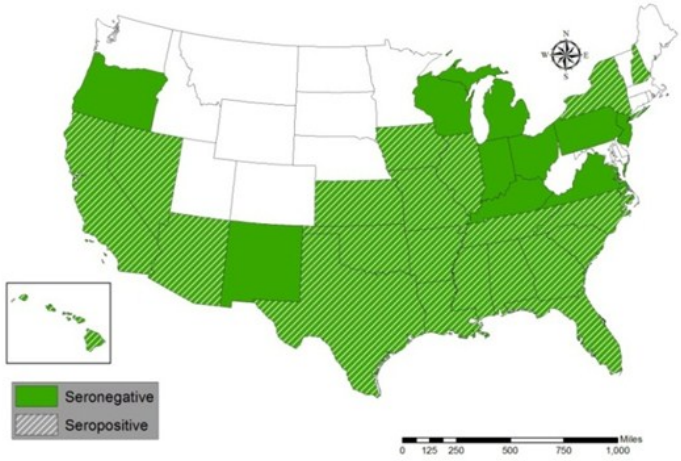


Figure 1: Distribution of seropositive and seronegative serum samples collected, by state.

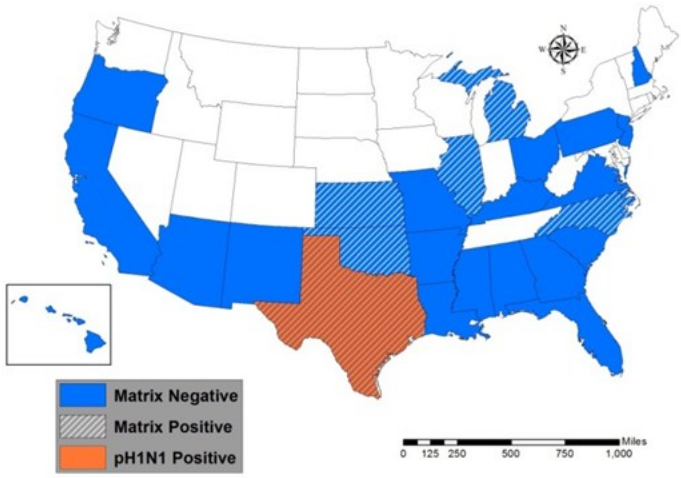


Figure 2: Distribution of matrix positive and negative nasal swab samples, by state.

Swine Influenza, continued

pose a threat to domestic livestock and human health.

Wildlife Services has cooperative agreements with various landowners and agencies to lethally remove feral swine that are causing damage to agriculture and natural resources. The NWDP opportunistically collects samples from these animals for disease surveillance. A paired nasal swab and serum sample are collected from each animal. Nasal swabs are placed into brain heart infusion media, refrigerated, and shipped to a predetermined NAHLN facility for influenza testing. The laboratories test nasal swabs for influenza A virus using real-time reverse-transcription polymerase chain reaction (rRt-PCR). All positive samples are further tested for the N1 neuraminidase using rRt-PCR and virus isolation. If virus isolation is positive, the sample is sequenced. Additionally, blood serum samples are aliquoted into cryogenic vials and shipped to the NWDP where they are tested for antibodies to influenza A viruses using the IDEXX b-enzyme linked immunosorbent assay test.

The NWDP began surveillance for Influenza in feral swine on 1November 2010. Sampling has been conducted in 31 of the 38 states with feral swine populations. As of 30 September 2011, the NWDP has sampled and submitted 1,895 nasal swabs and 2,501 serum samples for SIV testing. A majority of feral swine were sampled by WS personnel from January through March. The NAHLN laboratories have tested all 1,895 nasal swabs and 13 tested matrix positive by PCR. Further testing of the Influenza A positive samples resulted in two that demonstrated sequence homology to pH1N1. Of the 2,501serum samples, 256 (10.2%) were positive for Type A influenza antibodies.

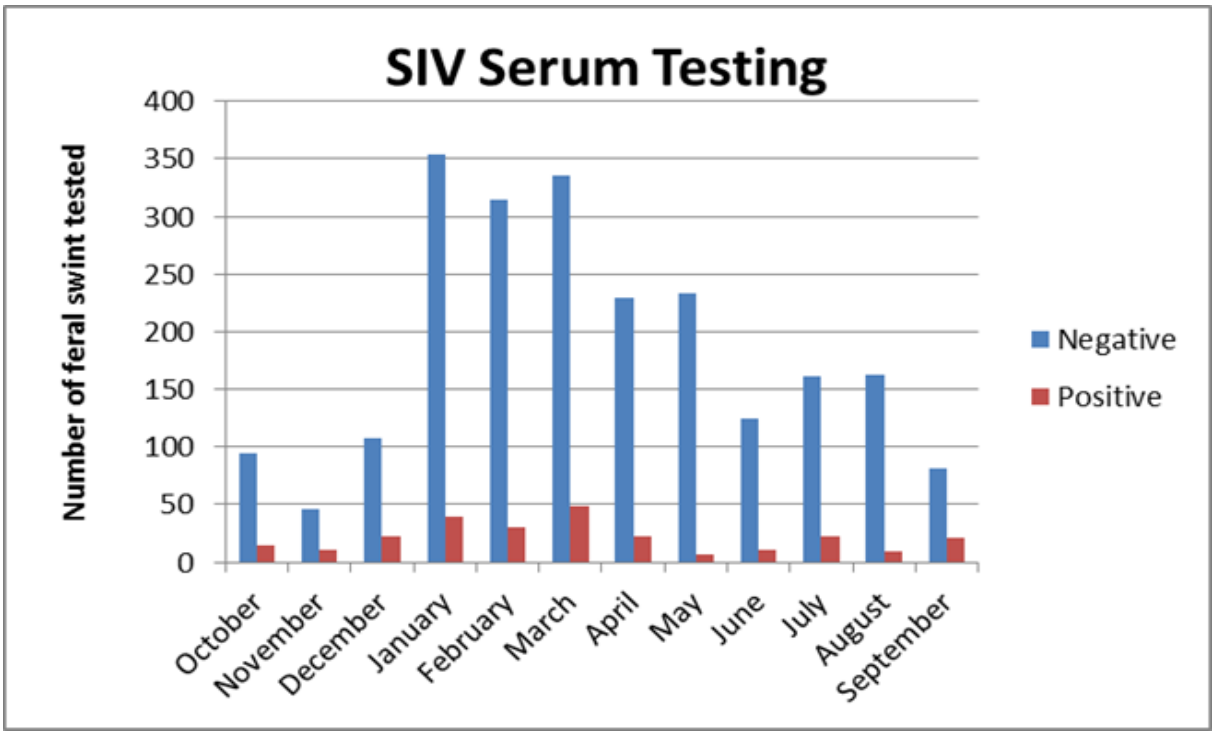


Figure 3: Distribution of SIV negative and positive feral swine samples, by month.

NWDP Surveillance Projects

Trichinella

Nematodes (roundworms) of the genus *Trichinella* are parasites of carnivorous and omnivorous animals. Transmission is through ingestion of muscle tissue containing encysted larval worms. When ingested, the larval worms leave the cyst and quickly mature into adults in the host small intestine. Adult parasites produce larvae, which migrate through the bloodstream to striated muscle tissue where they form cysts, completing the life cycle. Trichinellosis, the disease caused by *Trichinella* organisms, causes nausea, diarrhea, vomiting, fatigue and fever in people. These symptoms are usually followed by aching joints, muscle pain, rashes, itchy skin and weakness. If a high worm burden develops, heart function and breathing may be affected, and can result in death. Even in mild cases, weakness, fatigue and diarrhea may persist for months. In the early part of the 20th century, most human cases in the United States could be traced to pork. Domestic swine were commonly fed raw-meat garbage, and pork products were not always cooked adequately. Prior to World War II, an estimated 36% of people in the United States had contracted trichinellosis. The prohibition of feeding garbage to swine and education about the need to cook pork thoroughly has greatly decreased the incidence of trichinellosis in the United States. Today, relatively few cases (72 reported between 1997 and 2001) in people occur in the country, and most are associated with eating raw or undercooked wild game meats.

Figure 1: *Trichinella spiralis* larvae.

Trichinellosis is most commonly caused by the species *Trichinella spiralis*, which is especially well adapted to domestic swine and has a cosmopolitan distribution. There are at least three other endemic species of *Trichinella* found in wildlife in North America. Recently developed molecular biologic assays for larvae allow specific genotypes of *Trichinella* to be identified. It has been hypothesized that prior infection with *Trichinella* species effectively provides cross-immunity to swine from infection by the entire genus, and further, that *T. spiralis* will not be maintained in feral swine and other scavenging animals without the presence of infected domestic swine. The dynamics of parasite flow between domestic and feral swine is not well understood. With increasing demand for "organic" pork there is an increase in "pasture pig" operations in the United States. By regulatory definition, "organic" pork comes from domestic pigs that are allowed access to pasture at least once per day. Thus, the opportunities for interactions between domestic and feral swine are increasing.

Since 2009, the NWDP has been collecting 2,000 to 3,000 serum samples per year from feral swine to aid in identifying areas of potential risk for the domestic swine industry. Samples are submitted to the ARS Laboratory in Bethesda, Maryland where they are tested by enzyme linked immunosorbent assay for the

NWDP Surveillance Projects

Trichinella, continued

presence of antibodies to *Trichinella* species. Results indicate that *Trichinella* infect feral swine in 23 states (Figure 1). Although widespread, the apparent prevalence rate is fairly low, about 2%. The data also suggest clustering of positive cases. That is, when one infected swine is found others in the same sounder (family group) or the same general area tend to be infected as well.

In Fiscal Year 2011, the NWDP began collecting paired serum and tongue samples from feral swine in selected states and counties where previous sampling indicated exposure to *Trichinella*. The tongue, specifically the base of the tongue, is the preferred sample because *Trichinella* larvae have a predilection for this tissue. The larvae are extracted and genotyped to species. Serum from tissue positive *Trichinella* individuals will be examined to determine if species-specific markers are present in blood. In Fiscal Year 2011 wildlife disease biologists in four states collected and submitted 210 tongue samples. Of these, seven contained *Trichinella* larvae, which is consistent with the 2% apparent prevalence estimated by serology. Genotyping is proceeding and the results are pending.

This work will identify the species of *Trichinella* carried by feral swine as well as their immune status. Results will be useful in developing immunologically based strategies for protecting pasture-raised pigs from infection.

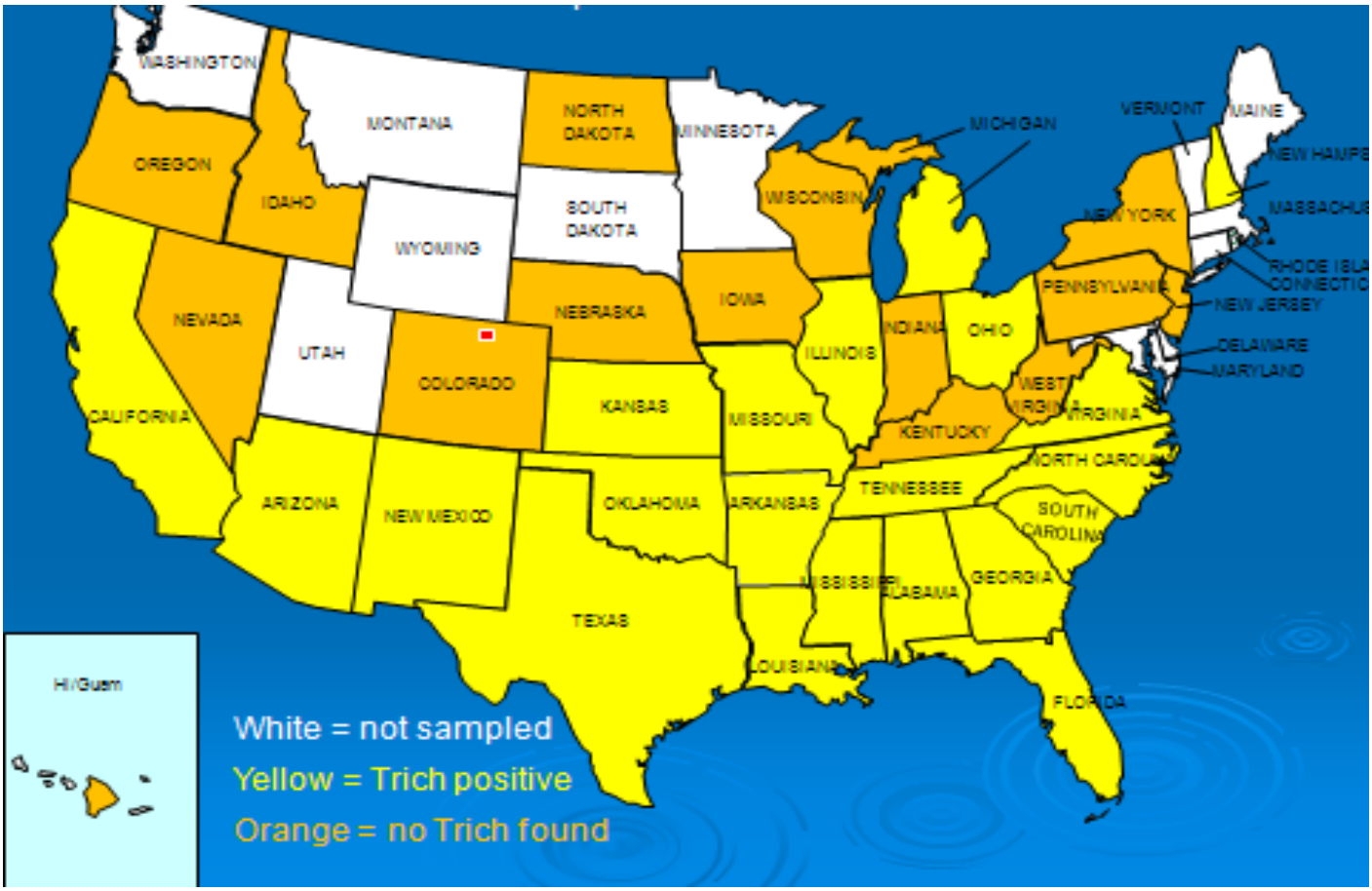


Figure 1: Results from *Trichinella* spp. surveillance in Fiscal Year 2010.

NWDP Surveillance Projects

Hepatitis E Virus

Hepatitis E Virus (HEV) is one of the five hepatitis viruses (A, B, C, D, and E) that can cause inflammation of the liver in humans. The HEV is a single stranded RNA virus in the genus *Hepevirus*. This disease is prevalent in most developing countries and is primarily spread through contaminated water supplies or consumption of undercooked meat. Since HEV is considered a waterborne disease, major outbreaks in humans are typically observed immediately following typhoons and heavy rains that result in flooding. There are four genotypes of HEV found throughout the world. Genotypes 3 and 4 are zoonotic, with domestic swine and several wildlife species (rodents, deer, feral swine) potentially serving as reservoirs. Recent studies have indicated that individual domestic swine operations may have infection rates as high as 95%.

The NWDP is collaborating with the NIH and the FDA to determine whether HEV is circulating in wildlife species and, if so, which genotypes are most prevalent. Surveillance for HEV will allow for both detection and identification of the genotypes circulating in feral swine and cervid populations. Paired samples (serum and fecal) are being collected to distinguish active shedding from exposure (seroprevalence).

Wildlife disease biologists are collecting samples from feral swine, cervids, and mongoose that are removed for wildlife damage management purposes. Whole blood is collected and centrifuged to harvest the serum, which is stored in 2 ml cryogenic vials and shipped to the NWDP office in Fort Collins, Colorado. Staff biologists prepare samples from the field and batch ship them monthly to the NIH laboratory for testing. Fecal samples are collected and placed in sterile re-sealable bags that are also shipped to NWDP. Fecal

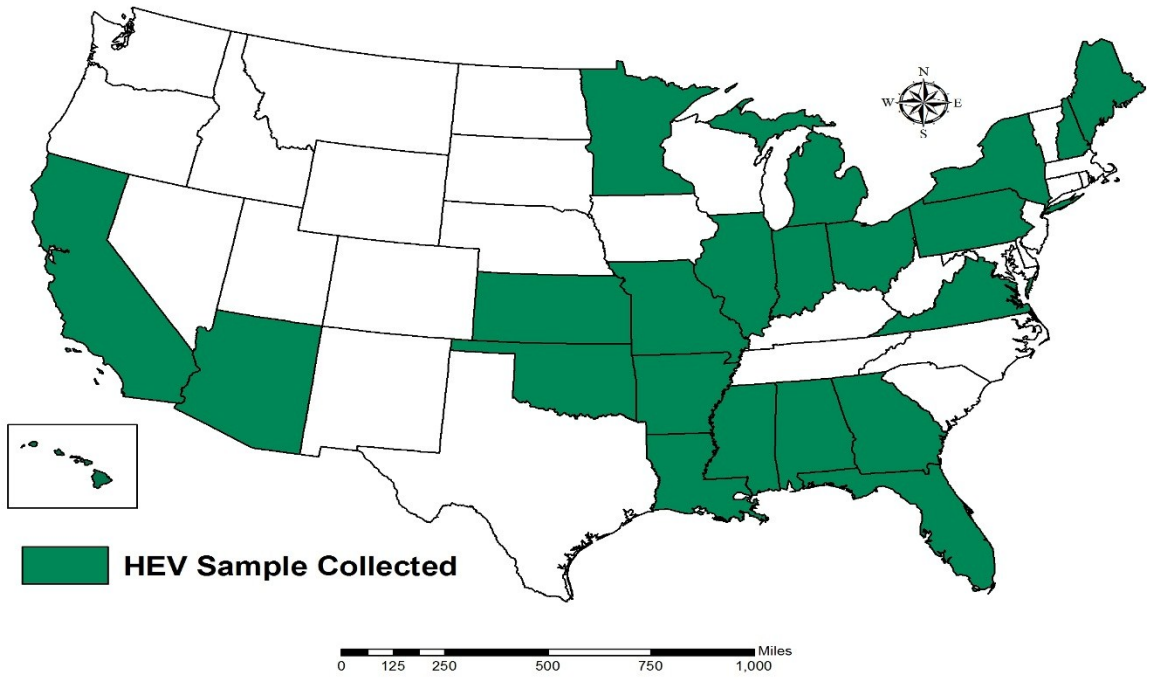


Figure 1: Distribution of Hepatitis E samples collected in Fiscal Year 2011.

NWDP Surveillance Projects

Hepatitis E Virus, continued

samples are batched and shipped monthly to a FDA laboratory where they are tested to determine if viral shedding is occurring.

The NWDP began surveillance for HEV in March, 2010. Sampling has been conducted in 22 states along with the United States and British Virgin Islands. A total of 845 samples were collected through 30 September 2011. One white-tailed deer from California and four feral swine in Hawaii were determined to be seropositive. Fecal sample testing is currently underway and results are pending.

Table 1: Number of samples collected for Hepatitis E surveillance, by state or territory, during Fiscal Year 2011.

State	Feral Swine	White-tailed deer	Indian Mongoose	Total
Alabama	42	74		116
Arkansas	3			3
Arizona	18			18
California	16			16
Florida	55	43		98
Georgia	50			50
Hawaii	43		11	54
Illinois		3		3
Indiana	2	8		10
Kansas	9			9
Louisiana	44	19		63
Maine		6		6
Michigan	8	55		63
Minnesota		114		114
Missouri	57	15		72
Mississippi	19	10		29
New Hampshire	14			14
New York	1			1
Ohio	4	2		6
Oklahoma	38			38
Pennsylvania	1	16		17
USVI & BVI		4	31	35
Virginia		10		10
Total	**424**	**379**	**42**	**845**

NWDP Surveillance Projects

Bluetongue Virus and Epizootic Hemorrhagic Disease

Bluetongue (BTV) and Epizootic Hemorrhagic Disease (EHDV) are devastating diseases of ruminants, including deer, cattle, sheep, and other species. It is estimated that BTV costs the United States' cattle and sheep industries $125 million annually and $3 billion world-wide. EHDV can have high mortality rates that can reduce local deer populations. These viruses are transmitted by several species of *Culicoides*, also known as biting midges or no-see-ums. Both viruses are found in the genus Orbivirus with numerous subtypes identified throughout the world. In the United States, there are six serotypes of BTV (1, 2, 10, 11, 13, and 17) and two serotypes of EHDV (1 and 2). Recent outbreaks of BTV-8 in Europe have prompted concerns that these foreign strains could enter the United States and have devastating impacts on livestock and wildlife.

Figure 1: Domestic sheep can be infected with Bluetongue Virus.

The NWDP is collaborating with the ARS to identify hot spots where EHDV/BTV outbreaks are occurring and establish surveillance around these locations. One objective is to identify the distribution/diversity of *Culicoides* species in these areas. A second objective is to test for EHDV/ BTV virus in Culicoides samples, and ultimately identify which species are vectors for the virus in each region.

Wildlife disease biologists identified trapping locations and set out CDC light traps in the evening and left them overnight to capture insects. The CDC light traps have an ultraviolet black light, as well as a canister of dry ice to emit CO^2 throughout the night. Insects were removed from the traps in the morning and immediately placed on dry ice. All insects were shipped to the NWDP office weekly during the

NWDP Surveillance Projects

Bluetongue Virus and Epizootic Hemorrhagic Disease, cont.

collection period, where they were sorted and identified. After identification, insects were tested using a multiplex real-time reverse-transcription polymerase chain reaction that can simultaneously screen for multiple serotypes of both BTV and EHDV.

Figure 2: Deer can also be infected with Bluetongue Virus.

The Fiscal Year 2010 trapping was conducted from May to September in Arizona, and June to September in Indiana. Indiana utilized six collection sites while Arizona went from four to five sites due to the diminishing water supplies in the area. Insects collected in Fiscal Year 2010 were processed and tested in Fiscal Year 2011. Samples collected in Fiscal Year 2010 produced 2,500 *Culicoides* from Arizona and almost 5,800 *Culicoides* from Indiana. Testing of the Arizona samples detected EHDV in *C. sonorensis* from two separate sites. BTV was detected in two species (*C. stellifer* and *C. biguttatus*) at two different sites in Indiana. Fiscal Year 2011 samples are in the process of being identified and will be tested for virus once this process is complete.

Table 1 : *Culicoides* species and sample sizes from Indiana collection sites.

Indiana	
Species	**Total # collected**
C. stellifer	2064
C. biguttatus	2776
C. verripennis	529
C. venustus	45
C. crepuscularis	287
C. haematapotus	29
C. obsoletus	22
C. piliferus	20
C. arboricola	20
Total	**5792**

Table 2: *Culicoides* species and sample sizes from Arizona collection sites.

Arizona	
Species	**Total # collected**
C. sonorensis	2250
C. crepuscularis	40
C. defoliarti	257
C. mojave	1
C. reevesi	2
C. selfia	8
C. butleri	1
Total	**2559**

NWDP Surveillance Projects

Toxoplasma gondii

Toxoplasmosis can be a serious disease in humans, affecting the developing fetus of women who acquire infection during pregnancy, and individuals who are immuno-compromised as a result of HIV-1 infection, lymphoma or immunosuppressive therapy. The causative agent is *Toxoplasma gondii,* a protozoan parasite with worldwide distribution. Humans can become infected with *T. gondii* by consuming undercooked meat carrying tissue cysts, or accidental ingestion of sporulated eggs (oocysts) from the environment, which are shed in the feces of felids (the cat family, Felidae). Currently about 12% of the United States population is thought to be infected with *T. gondii* and approximately 20% of deaths attributed to food-borne pathogens are due to *T. gondii* infection. Infection in healthy adults is controlled by the immune system, and rarely causes disease.

The incidence of *T. gondii* in enclosure-reared livestock, including swine, in the United States is low due to improved animal husbandry practices. However, *T. gondii* infection can be more common in free-ranging and backyard livestock. Most healthy livestock are resistant to clinical toxoplasmosis, but infections during pregnancy can cause abortions. Infection with *T. gondii* in wildlife is common, and in some species infection rates can be quite high (e.g., white-tailed deer, bear, feral swine, raccoons and bobcats), though they remain asymptomatic. *T. gondii* infection in wildlife species can be a source of infection for humans and livestock, and can be an indicator that *T. gondii* oocysts are present in the local environment.

Figure 1: Group of feral swine.

NWDP Surveillance Projects

Toxoplasma gondii, continued

The NWDP is collaborating with a variety of state and federal cooperators to learn more about the distribution, transmission and prevalence of *T. gondii* in wildlife. Researchers at the ARS, Animal Parasitic Diseases Laboratory in Beltsville, Maryland, with expertise in laboratory diagnostics and the epidemiology of toxoplasmosis, are key cooperators in the wildlife testing efforts, which support the broader goal of improving food safety in the United States

The primary objective is to survey feral swine and other species in the United States to determine the distribution and apparent prevalence of *Toxoplasma gondii*. When combined with habitat suitability models, the results can help predict the risk of future spread of *T. gondii* to new areas. A second objective is to determine the genotype of *T. gondii* in selected areas. Lastly, the study aims to find biomarkers in serum that correlate with genotypes of *T. gondii*, which would increase the utility of serosurveillance in the future.

At the national level, serology for *T. gondii* antibodies is performed with serum from feral swine sampled in 36 states. In Fiscal Year 2011 over 3,000 serum samples have been sent to the ARS. The collaboration has enabled spatial analysis of the distribution of *T. gondii* in feral swine. Based on these distribution maps, WS biologists in four states are now collecting selected tissues from feral swine in focal counties along with serum samples. *T. gondii* will be extracted from swine tongue tissue and used to inoculate mice. Mice will be sacrificed after 10 days and assayed for *T. gondii*, which will then be isolated for genotyping. The tissue collections will allow ARS scientists to isolate and identify different genotypes of *T. gondii.* This information will increase our understanding of the strains of *T. gondii* circulating in feral swine, and potential spillover between feral swine and pasture-raised, domestic swine.

Feral swine serum survey results from Fiscal Year 2011 are pending. In Fiscal Year 2010, 2,083 serum samples and 210 tongues were submitted to the ARS. Some samples are being re-tested to confirm the initial results. The apparent infection rate, 17.0 – 18.0%, appears similar to that of previous years. Positive serum samples were collected in 17 states, primarily in the south and southeast, and Hawaii. Overall, *T. gondii* positive serum samples have now been collected in 24 states. The distribution of *T. gondii* is thought to be restricted to regions with higher humidity, where cysts in tissue or oocysts in cat feces can remain viable longer before drying out. Results from the tissue extraction study are pending.

The ARS conducted diagnostic tests on the samples from Alaska. In addition to finding known *T. gondii* genotypes, a new atypical mouse-virulent genotype was isolated from one of the black bear samples collected near Alexander Lake in 2009. Such atypical genotypes are thought to be associated with cases of active toxoplasmosis in immunocompetent persons who are resistant to typical genotypes.

Initial results in Colorado include the isolation of *T. gondii* from four new bird species: barn owl, ferruginous hawk, rough-legged hawk, and Swainson's hawk. Continuing research goals include the genotyping of *T. gondii* isolates from a variety of wildlife species across the United States in collaboration with the ARS.

NWDP Surveillance Projects

Avian Influenza

Avian influenza viruses are classified based on two proteins, hemagglutinin (H) and neuraminidase (N), found on the surface of the virus. Specific viral subtypes have one of 16 different H proteins and one of nine different N proteins, resulting in 144 possible combinations. Each subtype can consist of numerous genetic sequence combinations that determine the pathogenicity (high or low) of the subtype to the infected host.

Wild birds, in particular certain species of waterfowl and shorebirds, are considered to be the natural reservoirs for all 144 subtypes of Influenza A viruses. These subtypes are adapted to survive in wild species and usually cause little or no disease; however, gradual genetic drift can occur resulting in a particular virus becoming capable of infecting other species of wild and domestic birds. Although this slight genetic change in the virus allows it to infect new species, it usually does not cause disease in the new host. The virus can also change if a host is simultaneously infected with another type A influenza virus. In such situations, mixing of the genetic material from the two virus strains (genetic shift) can occur, resulting in the formation of a new strain. The combination of gradual drifts and rapid shifts results in the production of strains that have the potential to cause morbidity and mortality in susceptible hosts. If morbidity and mortality are significant, the virus is classified as a highly pathogenic avian influenza (HPAI) virus.

Highly pathogenic avian influenza subtypes can be devastating to the poultry industry and be a potential threat to human health. While HPAI does not currently exist in the United States, it could be introduced via illegal movement of domestic or wild birds, in contaminated products, by infected travelers, through bioterrorism, and during the migration of infected wild birds. Surveillance for HPAI in wild birds was initiated in the United States in 2006 in response to detections of the virus in wild birds in Asia and Europe. There was concern that wild birds would introduce the virus during migration which could be detrimental to the poultry industry or potentially result in human morbidity or mortality. Although the Pacific Flyway was initially thought to be the likely site of introduction, surveillance was conducted in all four North American flyways to ensure the goal of early detection in wild, migratory birds.

The primary objective of the surveillance system was early detection of any HPAI virus and provide early warning to the poultry industry and public health officials. An emphasis was placed on active surveillance of ducks, geese, and shorebirds and passive surveillance of all birds through investigating morbidity/ mortality events. A secondary objective was to characterize the low pathogenic avian influenza viruses circulating throughout the United States The sampling effort was also successful in establishing that the United States is free of highly pathogenic avian influenza (HPAI) and fostered cooperative relationships between federal, state and tribal wildlife agencies and universities.

Surveillance was initiated on 1 April of each year and ended the following 31 March . Each state was given a ranking to allocate samples based on several factors including amount of wetland habitat, overwintering waterfowl population size, linear distance of shoreline, and waterfowl band recovery data. The ranking (1, 2 or 3) corresponded with the number of samples that each state would collect. Surveillance was targeted

Table 1: Total wild bird samples collected by biological year.

BY2006	BY2007	BY2008	BY2009	BY2010
85,501	62,788	64,741	44,621	25,709

Avian Influenza, continued

towards competent H5 or H7 carriers and groups of species with similar biological classifications and feeding habits (dabbling ducks, divers, geese & swans, etc.) were used to target species for sampling. Investigation and subsequent sampling of all birds discovered dead was encouraged. A number of methods for collecting samples were utilized including live capture and release, hunter harvested birds, sentinel birds, environmental sampling and investigation of morbidity/mortality events. Environmental sampling was discontinued in 2009.

During Biological Year 2010, the target number of samples was 44,000. However, on 30 September 2010 sampling for WS employees was discontinued due to lack of funding. Despite this difficulty, state wildlife agencies continued to collect samples through 31 March 2011 under cooperative agreements established in Fiscal Year 2010.

One cloacal and one oropharyngeal sample were collected from each bird and placed in a tube of transport media. The transport media consisted of 3 mL of brain heart infusion broth in cryogenic vials and was provided to all collectors by the NVSL to ensure standardization. Samples were shipped to a laboratory that

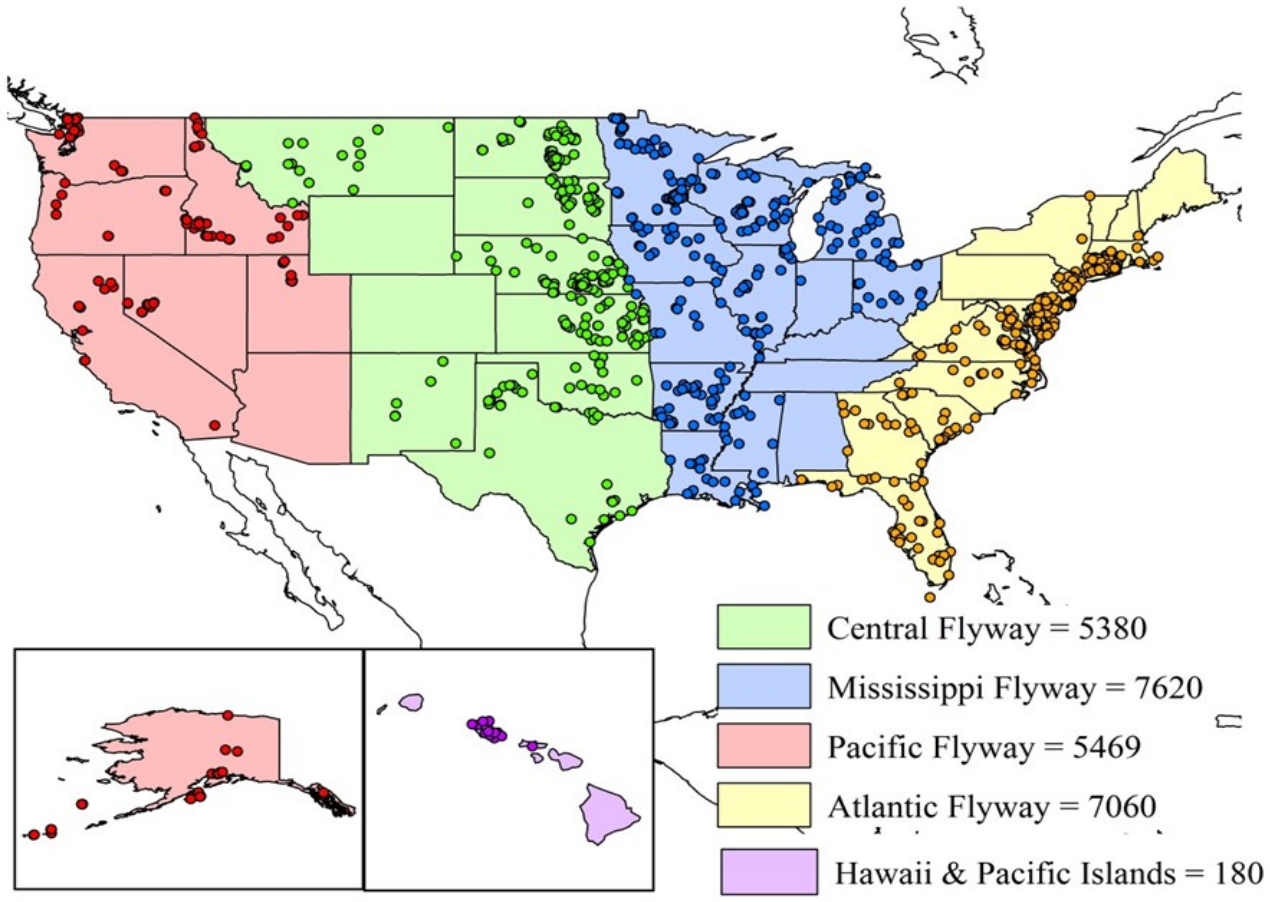


Figure 1: Avian influenza sample collection sites by flyway for Biological Year 2010.

Avian Influenza, continued

is part of the NAHLN within 48 hours of collection. The NAHLN laboratories screened the samples within 48 hours of receipt with the matrix gene real-time reverse-transcription polymerase chain reaction assay. Testing was considered complete on samples testing negative but positives samples were further tested with both H5 and H7 specific real-time reverse-transcription polymerase chain reaction assays. Samples testing positive on either assay were forwarded to the NVSL for virus isolation and pathogenicity testing.

During Biological Year 2010, 25,709 samples were collected across the United States Samples were distributed across the Atlantic, Central, Mississippi, and Pacific flyways as well as American Oceania (Figure 1).

Table 2: Number of matrix positives, by state, for Biological Year2010.

State	# of samples collected	# of matrix positive samples
Alabama	0	0
Alaska	1516	190
Arkansas	884	147
Arizona	0	0
California	608	69
Colorado	0	0
Connecticut	963	58
Delaware	866	86
Florida	713	49
Georgia	469	25
Hawaii	180	0
Iowa	851	191
Idaho	506	149
Illinois	648	104
Indiana	49	0
Kansas	596	83
Kentucky	0	0
Louisiana	750	66
Massachusetts	24	1
Maryland	771	99
Maine	0	0
Michigan	1246	229
Minnesota	1013	215
Missouri	515	112
Mississippi	546	168

State	# of samples collected	# of matrix positive samples
Montana	834	251
North Carolina	531	11
North Dakota	690	138
Nebraska	1076	105
New Hampshire	2	0
New Jersey	835	74
New Mexico	376	40
Nevada	759	152
New York	390	4
Ohio	330	31
Oklahoma	413	30
Oregon	661	59
Pennsylvania	0	0
Rhode Island	0	0
South Carolina	686	32
South Dakota	701	233
Tennessee	0	0
Texas	694	91
Utah	447	86
Virginia	810	23
Vermont	0	0
Washington	972	324
Wisconsin	788	263
West Virginia	0	0
Wyoming	0	0

NWDP Surveillance Projects

Baylisascaris

The disease producing capability of nematodes in the genus *Baylisascaris* have become an increasing health concern during recent years. Within North America, there are four species in the genus *Baylisascaris* of concern in regards to their capacity to cause disease. The two species of interest to the NWDP are *Baylisascaris procyonis* and *Baylisascaris columnaris*, because they are the most common cause of zoonotic disease in both animals and humans, and utilize common species such as raccoons and skunks as definitive hosts.

The main species of concern is *Baylisascaris procyonis*, a large intestinal nematode commonly found in raccoons throughout the United States. *B. procyonis* is the most commonly recognized cause of clinical larva migrans in both animals and humans and is best known as a cause of fatal or severe neurologic disease. Over 90 species of birds and mammals are susceptible to *B. procyonis* infections resulting in high morbidity and/or mortality rates. Highly susceptible intermediate hosts, include various rodents, lagomorphs, and avian species. In intermediate hosts the larvae aggressively migrate through the visceral and neural tissues.

Intermediate hosts frequently become infected by foraging among communal raccoon latrine sites, which provide granivorous birds and mammals a readily available cache of undigested seeds. In addition to ingesting infective *B. procyonis* eggs while foraging, infections can also occur from routine grooming or drinking contaminated water, as these eggs readily adhere to multiple surfaces and can survive in the environment for multiple years depending on temperature and humidity.

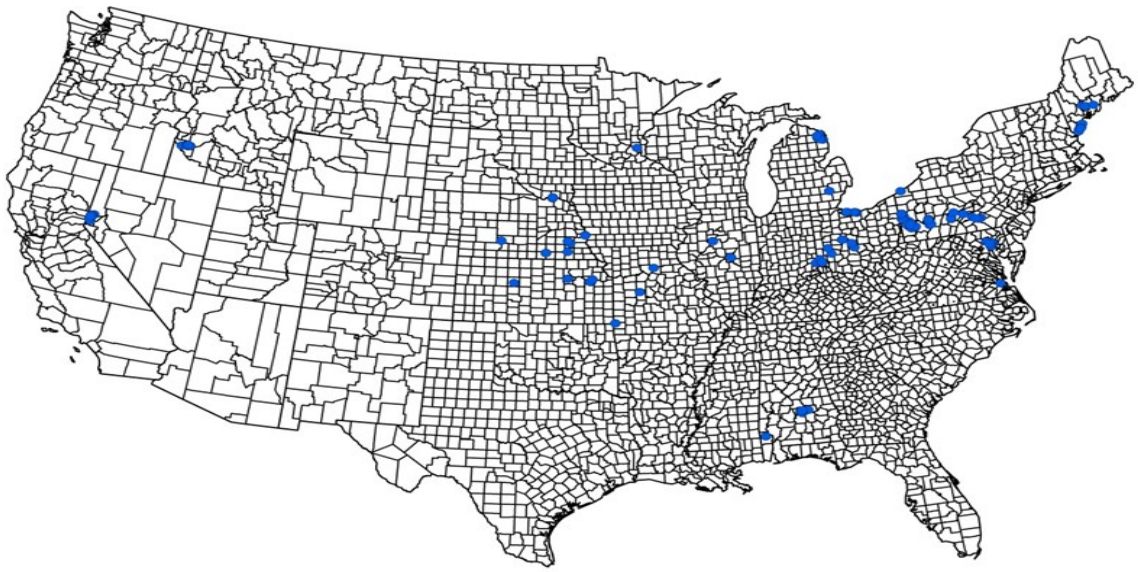


Figure 1: Distribution of samples submitted for *Baylisascaris* spp. surveillance to date.

NWDP Surveillance Projects

Baylisascaris, continued

Table1: Number of samples submitted during Biological Year 2011.

State	Species	Number of samples
Alabama	Raccoons	40
	Skunks	-
Idaho	Raccoons	16
	Skunks	2
Illinois	Raccoons	26
	Skunks	3
Indiana	Raccoons	27
	Skunks	-
Kansas	Raccoons	19
	Skunks	8
Maryland	Raccoons	38
	Skunks	-
Maine	Raccoons	19
	Skunks	12
Michigan	Raccoons	32
	Skunks	-
Minnesota	Raccoons	3
	Skunks	-
	Other	1
Missouri	Raccoons	22
	Skunks	-
Nebraska	Raccoons	37
	Skunks	-
Nevada	Raccoons	20
	Skunks	4
Ohio	Raccoons	66
	Skunks	-
Pennsylvania	Raccoons	57
	Skunks	7
Virginia	Raccoons	64
	Skunks	-
Wyoming	Raccoons	-
	Skunks	1
Total	Raccoons	486
	Skunks	38

Although *B. procyonis* infections in humans are relatively rare, this parasite is an important zoonosis because the disease is often very severe or fatal, is difficult to diagnose, lacks an appropriate treatment, and typically occurs in young children or persons with learning disabilities (due to increased likelihood of ingesting fecal contaminated dirt or foreign objects). Currently there is no highly effective treatment for larval migrans associated with *B. procyonis* in humans. The lack of commercially available serologic tests for large-scale epidemiologic studies has limited our understanding on how common mild cases are within the human population.

The objectives of the NWDP monitoring of *Baylisascaris* in wildlife are to determine the apparent prevalence and distribution of *B. procyonis* in raccoons and *B. columnaris* in skunk populations at a national scale; to identify and document factors associated with *B. procyonis* expansion; correlate human cases with infection rates in wildlife and domestic animals; and compile risk assessment maps for humans living in endemic areas.

Raccoons and skunk are being trapped in conjunction with several other disease projects including surveillance for rabies, plague, and tularemia. Intestinal tracts from these animals are extracted, double bagged, frozen, and then submitted for parasite surveillance at the NWDP office. Once an intestinal tract has been examined and found to be positive for intestinal parasites, those parasites are then examined via microscopy and identified morphologically when applicable, or are set aside for genetic analysis using a polymerase chain reaction test.

Surveillance was initiated on 1 June 2011 and will continue until 30 September 2012. To date 524 samples have been received and are being screened for the presence of parasites.

NWDP Surveillance Projects

Plague

Yersinia pestis is a flea-borne bacterium and the agent responsible for plague. The pathogen is traditionally described as cycling through small mammal populations, with an enzootic cycle and an epizootic cycle. The enzootic cycle of plague is maintained among rodent hosts and their fleas; however, transmission to humans and other mammals can occur through flea bite or direct contact and, in some cases, results in severe morbidity and mortality.

A majority of human plague cases in the United States are associated with peridomestic transmission in non-urban areas, often involving bites from rodent fleas or even pneumonic transmission from contact with domestic pets. From 1950 through 2009, 464 plague cases were reported in the United States. Despite this relatively limited occurrence in humans, evidence of plague exposure in regions of the Western United States in non-domestic rodents and carnivores is substantial. This is likely because plague is essentially a disease of rodents, many of which survive plague exposure or have limited to no clinical symptoms at all. Carnivores that prey upon these rodents, or are exposed to their fleas, can also be exposed to the bacterium.

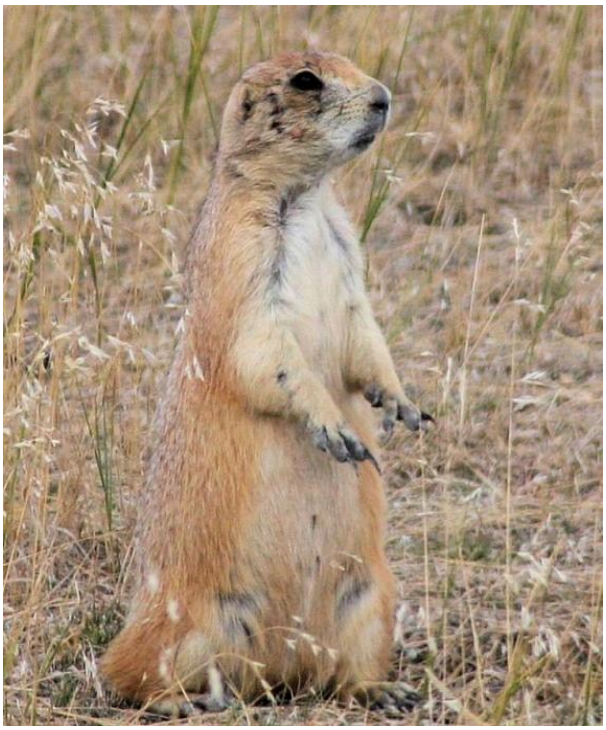


Figure 1: Plague can cause significant mortality in prairie dogs.

Like other areas in the world, plague activity in the United States is often difficult to detect for extended periods of time. There is typically limited evidence of regular plague transmission, but these low activity periods are interspersed by occasional epizootics that result in highly visible die-offs of some rodent species. Prairie dogs suffer the most dramatic die-offs in the United States, with plague affected populations suffering up to 98% mortality. While documenting these die-offs is an efficient and low-cost way to monitor plague dynamics, they often are detected only after an epizootic has been underway and may not necessarily serve as an early warning system.

Monitoring plague exposure, or seroprevalence, through active surveillance of other animals, such as coyotes that can act as sentinel species, is a viable option for monitoring plague dynamics. The objective for this surveillance project is to determine plague exposure in wildlife, on a national scale, to better understand transmission dynamics and risk of human exposure.

The NWDP has directed a long-term plague surveillance program, in cooperation with the CDC, the Washington State Health Department, the Texas Department of Health, and other local and tribal agencies in the United States. *Yersinia pestis* has not colonized the eastern half of the country since its introduction to the United States at the beginning of the 20th century and surveillance efforts are primarily restricted to areas west of the 100th meridian. Plague surveillance by the NWDP is conducted through opportunistic sampling of wildlife species, with a focus on sentinel species, such as coyotes. Blood samples are collected

Plague, continued

on Nobuto strips by wildlife disease biologists and other WS personnel and disseminated to the CDC for screening. Samples have been collected from multiple species and across large spatial scales since 2005, although testing levels were reduced by the CDC in 2011.

To date, 46,801 samples have been collected from 2005-2011 (Figure 1) as part of the *Y. pestis* surveillance program. Subsets of these samples (n=28,948) were chosen for plague screening based on geographic location, and included samples collected from multiple species across 29 states (Table 1, Figure 2). Overall there was substantial exposure to Y. pestis in wildlife in the western United States, with the highest apparent seroprevalence rates in coyotes (mean=10.75%). States with the highest apparent exposure levels across all species were New Mexico (mean=21.04%) and Wyoming (mean=25.10%).

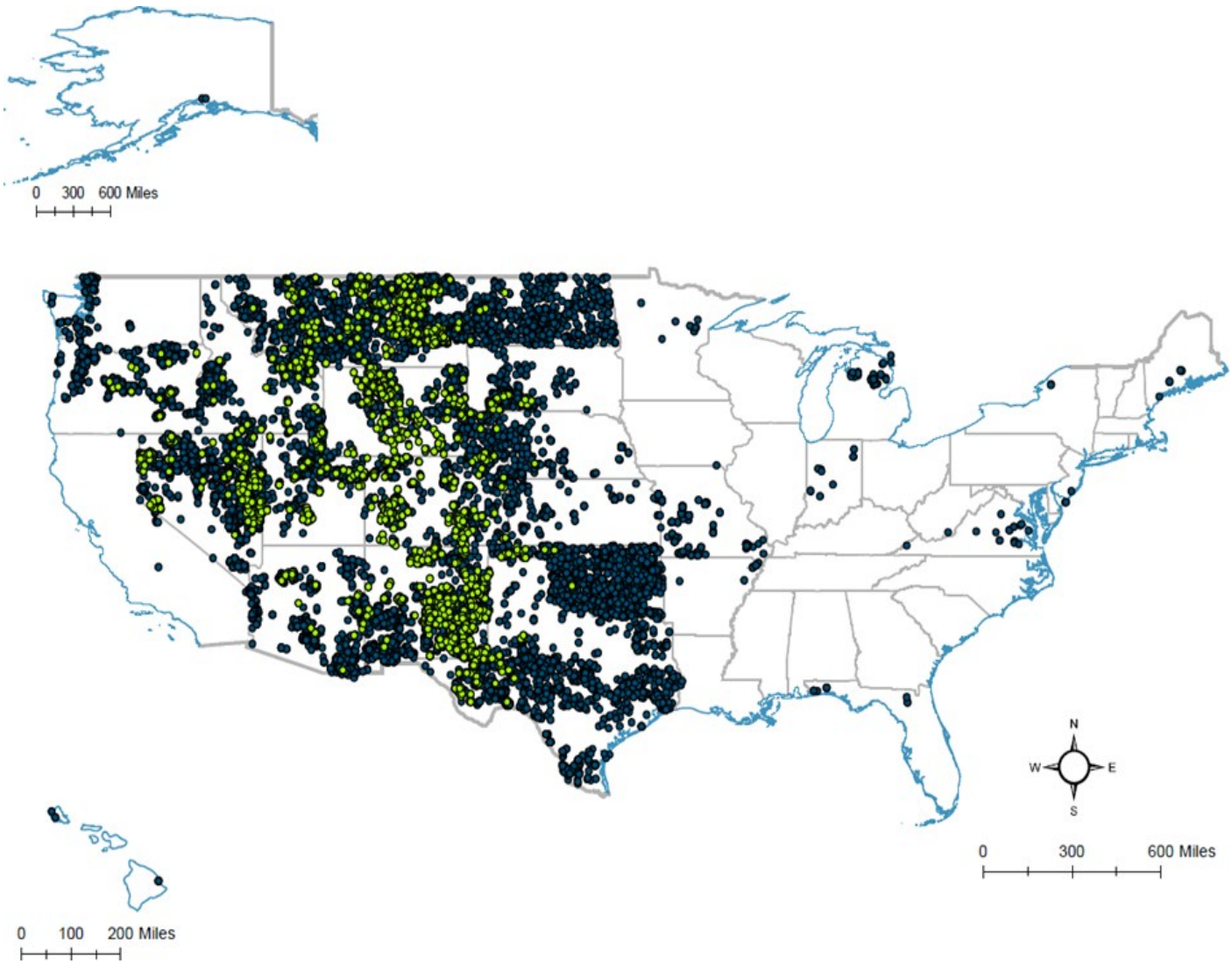


Figure 2: Plague surveillance in wildlife, 2005-2011. Blue symbols represent a *Y. pestis* negative sample; green symbols represent a *Y. pestis* positive sample.

NWDP Surveillance Projects

Tularemia

Tularemia is a highly infectious zoonotic disease caused by the bacterium *Francisella tularensis*. First described in the early 1900's and initially isolated in Tulare County, California, *F. tularensis* has now been separated into four subspecies based on geographic occurrence and ecological associations; however *F. tularensis tularensis* (Type A) and *F. tularensis holarctica* (Type B) are the subspecies most often associated with human disease. Both are found in the United States, but have different geographic distributions. Tularemia is considered to be one of the most infectious pathogens ever studied; its ability to readily aerosolize and potential use as a bioterrorism agent make it part of the National Notifiable Diseases Surveillance System.

Figure 1: Rabbit die-offs can be associated with tularemia.

More than 200 species have been documented with naturally occurring *F. tularensis* infections and multiple vectors, including ticks and tabanid flies, are thought to be involved in transmission; however, only a few vectors and host species are thought to play an important role in perpetuating the pathogen. *Francisella tularensis tularensis* typically cycles in terrestrial environments and is primarily associated with lagomorphs and arthropod vectors. Flies and mosquitoes are thought to be mechanical vectors, while ticks are biological vectors and may also potentially act as a reservoir. *Francisella tularensis holarctica* is associated with aquatic environments, particularly rodent species. Approximately 100-150 human tularemia cases occur every year in the United States and most are attributed to tick bites, although outbreaks linked to aerosolized tularemia, biting-flies, and mosquitoes, have been documented as well. Human infections can result in severe clinical symptoms, including fever, localized ulcer at cutaneous inoculation site, and respiratory distress if exposed through inhalation, although infections are rarely fatal when treated with antibiotics.

By monitoring tularemia exposure, or seroprevalence, in wildlife we can better understand the ecological dynamics governing tularemia in the United States. The NWDP, in conjunction with animal damage management activities or other research projects conducted by WS, opportunistically obtains samples from multiple species from across the country to screen for tularemia exposure. Samples are collected on Nobuto blood filter strips, in cooperation with state and other federal agencies, and screened at the CDC using standard protocols.

Tularemia, continued

To date, 46,801 samples have been collected from 2005-2011 (Figure 1) as part of the *Y. pestis* and *Francisella tularensis* surveillance program. Subsets of these samples (n=20,727) were chosen for tularemia screening based on geographic location, and included samples collected from multiple species across 46 states (Table 1, Figure 1). Tularemia exposure was very low overall, although when it was identified, it was widely spread across states and species. Illinois (mean apparent seroprevalence = 4.54) and Missouri (mean apparent seroprevalence = 2.57) had the highest occurrence, relative to other states; however, positive sample sizes were still very low.

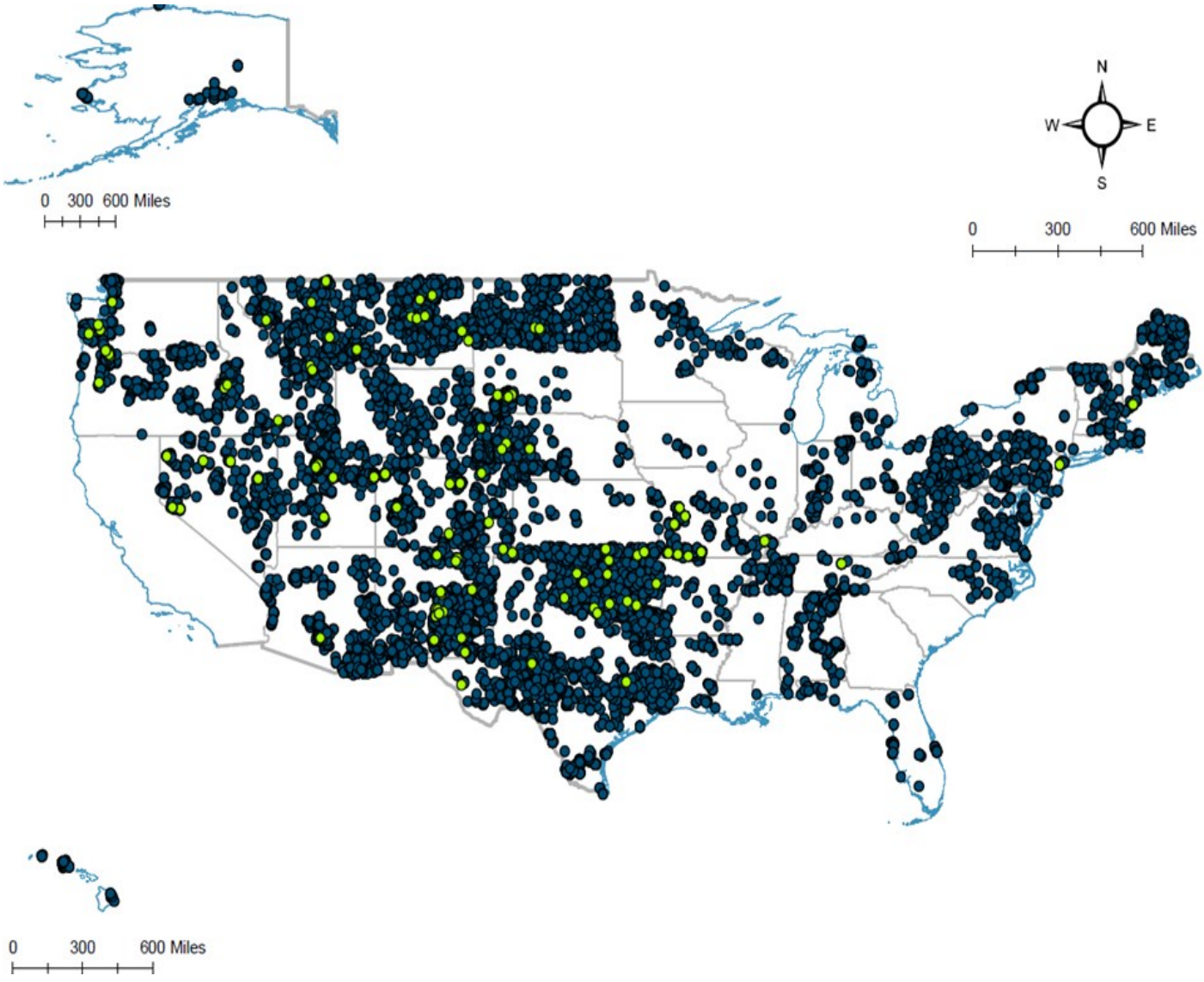


Figure 2: Tularemia surveillance in wildlife, 2005-2011. Blue symbols represent an *F. tularensis* negative sample; green symbols represent an *F. tularensis* positive sample.

NWDP Surveillance Projects

Neospora caninum

Figure 1: *Neospora caninum* can cause abortion in pregnant cattle.

In Fiscal Year 2011, the NWDP initiated a retrospective surveillance project to determine wildlife exposure to *Neospora caninum*. This project utilizes Nobuto blood filter strips from the Plague and Tularemia Sample Archive, as well as serum samples stored in the Feral Swine Serum Archive. The specific objectives of this project are to determine if coyotes and feral swine are being exposed to the parasite, and to develop insights on the potential links between zoonotic transmission dynamics and livestock.

Neospora caninum is an intracellular, apicomplexan parasite that is similar in many ways to *Toxoplasma gondii*. Both are coccidian parasites, and both produce environmentally resistant, infective oocysts that are passed through the digestive system of definitive hosts as part of a complex reproductive cycle; however, while the definitive hosts for *T. gondii* are felids, the only definitive hosts for *N. caninum* that have been identified to date are canids, including coyotes, domestic dogs, wolves, and dingos. *Neospora caninum* was first identified in 1988 and the list of canid definitive hosts is likely to increase as additional research is completed. Examining *N. caninum* exposure in coyotes, which are likely a key definitive wildlife host in western states, will important information on the ecology of this parasite. Previous research has indicated a 10-11% exposure rates in limited numbers of coyote samples. To date, there is no information on *N. caninum* exposure in populations of feral swine in the United States, or if they have similar rates of exposure as cattle and sheep. In Europe, an 18.1% exposure rate in wild boar has been reported.

The primary interest in this organism stems from the isolation of *N. caninum* in cows, where it is the most frequently diagnosed cause of abortion in both dairy and beef cattle. Transmission in cattle is primarily vertical, with positive females passing the infection to calves. Epidemic outbreaks have been documented where > 50% of dairy cows in a herd abort within several weeks of each other, but losses in sporadic or endemically infected herds may be much lower because of an unpredictable recrudescence rate in infected cows. Infected adult cattle generally exhibit no clinical signs.

Limited information exists on economic losses associated with *N. caninum*, but one study estimated that the loss in California alone approached $35 million per year and a study in Texas estimated losses between $15 and $24 million per year. Economic losses stem from reproductive problems and abortion, loss of milk yield, reduced growth, and decreased feeding efficiency. In addition to cattle, *N. caninum* can cause clinical disease is dogs, as well as in sheep and goats, although the epidemiologic importance in these species remains unknown.

The NWDP surveillance project is focusing on overlapping populations of coyotes and feral swine (i.e., Texas, Oklahoma, and New Mexico) in order to capture disease dynamics in both a definitive host and an incidental host. Serologic surveys and DNA isolation suggest that a large number of domestic and wild mammals are exposed to *N. caninum*, but the parasite has only been successfully isolated from a few species such as cattle, sheep, white-tailed deer, and bison.

Newcastle Disease

Figure 1: Domestic poultry operations can suffer high mortality rates during a Newcastle Disease outbreak.

Newcastle disease (NDV) is a contagious viral disease affecting birds. Understanding this disease is crucial to the poultry industry because it causes illness, death and reduced egg production resulting in severe economic losses. In wild birds, the effect appears to vary depending on the species of bird and the virulence of the particular NDV strain. More than 230 species of birds have been found to be susceptible to natural or experimental infections with avian paramyxoviruses. Mortality due to NDV infection have been reported in cormorants, white pelicans, ring-billed gulls and California gulls.

Newcastle disease is caused by infection with an RNA virus within the avian paramyxovirus-1 group. Virulence of NDV varies with the strain of the virus and is measured by its ability to cause disease in chickens. Velogenic, or highly virulent Newcastle disease virus, are the most virulent strains and cause acute, lethal infection in chickens that can result in 100% mortality. These strains produce hemorrhagic lesions in the digestive tract. While rare in the United States, these viruses have been introduced on occasion, through illegal trafficking of exotic bird species. Neurotropic Newcastle disease is another virulent form of NDV that is generally lethal and affects respiratory and neurologic tissues. Morbidity is

Newcastle Disease, continued

usually very high, but mortality is much lower than velogenic NDV. Mesogenic strains of NDV cause neurologic signs, but mortality is low. Lentogenic strains cause mild or unapparent respiratory infections in chickens, although some strains cause asymptomatic-enteric infections without visible disease.

While a national surveillance program has not been initiated for NDV, the NWDP encourages WS' wildlife disease biologists to investigate all morbidity/mortality events. The NDV is usually suspected when mortality rates are high and species such as cormorants, gulls, or pelicans are detected in regions where outbreaks frequently occur, such as the upper Midwest or northeast regions. For smaller scale mortality events (<500 birds), wildlife disease biologists are encouraged to submit samples to their state diagnostic laboratory and/or the NWHC in Madison, Wisconsin. For larger scale mortality events (>500 birds), wildlife disease biologists are advised to submit samples directly to the NVSL. The NWDP also collaborates with the SEPRL in Athens, Georgia on developing a wild-bird specific assay for detecting NDV. Once NDV has been confirmed at another laboratory, SEPRL requests additional samples to validate the assay. In most cases, individual cloacal and oropharyngeal samples are collected and combined in a single 3 mL vial of brain heart infusion broth. Depending on the where the samples are shipped, the entire carcass or specific organs may be submitted for testing. Results are reported back to the local office. Virulent NDV was detected in Maine, New Hampshire, and Massachusetts during Fiscal Year 2011.

In addition to morbidity and mortality event investigations, the NWDP also collaborates on specific NDV research projects. During Fiscal Year 2011, wildlife disease biologists from Minnesota and Wisconsin collaborated with the NWHC to collect samples for a project to learn more about NDV in cormorants and gulls. Wildlife disease biologists from Michigan, Rhode Island, New York and New Jersey also collected samples as part of a mute swan surveillance project aimed at learning more about diseases and pathogens carried by mute swans (see Mute Swan Surveillance Project).

Figure 2: Areas where multiple species congregate can be sampled as part of disease surveillance activities.

Cooperative Activities

Avian Bornavirus

Borna Disease is a neurological disease that primarily affects horses, but also sheep, goats, llamas, cattle, dogs, domestic cats, rabbits, deer, and lynx. It can cause fatal neurological and behavioral disorders in warm-blooded animals. Infection with Borna Disease Virus (BDV) has been associated with some psychiatric disorders in humans. BDV is a non-segmented negative strand RNA virus (Order Mononegavirales; Family Bornaviridae). The name comes from the Saxon (German) city of Borna where the disease was common in the early 20th century, resulting in the deaths of more than 16,600 horses between 1896 and 1940. At least one confirmed natural host is the bicolored white-toothed shrew. Virus is shed in the shrew's urine. Other wildlife hosts have been proposed, but not confirmed. BDV appears to be globally distributed and, recently, it was determined that gene sequences related to BDV are incorporated into the human genome, as well as those of many other mammals and fish. This suggests a long association of BDV with vertebrate species.

Avian Bornavirus (ABV) is the etiological agent of proventricular dilation disease (PDD) in psittascine birds (parrots, macaws, conures). PDD is characterized by damage to the nerves of the enteric system. Food accumulates in the paralyzed proventriculus, eventually leading to death. Although PDD disease was first described in the United States in captive parrots and macaws during the late 1970's, until now no

Figure 1: Bornavirus infections have been identified in symptomatic Canada Geese.

Cooperative Activities

Avian Bornavirus, continued

natural reservoir of BDV had been found in North America. In response to reports of two Canada geese in Prince Edward Island, Canada, displaying PDD-like neurological signs and lesions, researchers at the Texas A& M University, Department of Veterinary Pathology, speculated that waterfowl might be a possible reservoir. Borna-infected birds are known to intermittently shed virus in the feces. A study was initiated when researchers contacted the NWDP to request access to cloacal/oropharyngeal swab samples from the Wild Bird Tissue Archive.

The objective was to retrospectively examine cloacal/oropharyngeal swabs from Canada geese for the presence of ABV, using a real-time reverse-transcription polymerase chain reaction. Also, brain tissue of apparently healthy, hunter-harvested Canada geese was assayed, and positive samples were isolated and sequenced.

In late Fiscal Year 2010, the Wild Bird Tissue Archive staff supplied over 400 Canada goose samples representing 12 states and all four United States migratory flyways. In addition, NWDP wildlife disease biologists in New Jersey and Kansas submitted heads of apparently healthy, hunter-harvested or nuisance Canada geese and Lesser Snow geese for testing. At the Texas A&M Veterinary Diagnostic Laboratory, RNA was purified from swab samples, and cDNA was generated from brain tissues. Brain isolates were cultured in duck embryo fibroblasts and sequenced. To demonstrate that the results were not due to artifacts from laboratory contamination, the procedures were repeated at a second laboratory with no known history of BDV exposure.

Bornavirus sequences were detected in 12/409 swab samples (approximately 2.9%). Positive samples came from five states in three flyways (Atlantic, Mississippi and Pacific). Bornavirus was also detected in 13/25 (52%) of brain samples. Findings suggest that bornavirus might be quite common in apparently healthy Canada geese, but more sampling must be conducted to support this conclusion. The lower rate of detection in swab material is not unexpected, since studies of infected captive birds have shown that fecal shedding of detectable levels of ABV is intermittent.

Phylogenetically, the Canada goose brain isolates formed a tight cluster distinct from the psittacine ABV. In fact, the Canada goose isolates were more similar to the mammalian BDV than to avian bornavirus. They also align closely with a previously recovered sample from an encephalitic Canada goose. Similar analyses of brain tissue isolates from Lesser snow geese in Kansas and Mute swans in Michigan have also been conducted. Results are pending. The grazing habits of geese are conducive to disease spread within flocks by the fecal-oral route, which has been identified as a likely mode of transmission.

The results of this study suggest that bornavirus infection may be fairly common in apparently healthy Canada geese and clinical infection appears to be rare, suggesting that they may serve as reservoirs. However, their ability to transmit the disease to other animals remains unknown. Future research will try to elucidate the host range among birds and small mammals, determine modes of transmission, and the frequency of clinical infections in wildlife.

Disease in Mute Swans

Figure 1: Mute swans

Mute swans are considered an invasive species in many parts of the United States. They are prolific birds that are not typically afraid of humans, which can lead to large swan population densities in many urban areas. They cause significant ecological damage by eating submerged aquatic vegetation that serves as food for native waterfowl and habitat for fish and crabs, and their aggressive nature can prevent other birds from nesting, as well as threaten human safety. Some WS programs in the Great Lakes region received funding from the Great Lakes Restoration Initiative to lethally remove mute swans with the goal of minimizing ecological damage. The NWDP wildlife disease biologists in this region and in the northeast have been taking advantage of the swan removal by opportunistically collecting samples for disease surveillance.

The NWDP set up collaborative projects to look at specific diseases with Michigan State University, ARS, SCWDS, and Texas A&M University. One of the primary diseases of concern is Newcastle disease virus (NDV) which can affect many domestic and wild birds. There are many die-offs that occur in the Great Lakes region and northeastern United States that are attributed to NDV. If mute swans are infected they may transmit the disease to other susceptible wild birds or poultry.

Other pathogens of concern include the *Schistosoma* parasites, one of which is the cause of swimmer's itch

Disease in Mute Swans, continued

in humans. The intestinal tracts of swans were also examined for *Salmonella* and *Toxoplasma*. *Salmonella* are ubiquitous in nature but in most cases the serotypes are species specific, although there are a few serotypes capable of causing disease in various species including humans. Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii*, a protozoan that infects most mammals, including humans. Samples were collected to evaluate their exposure to *T. gondii.*

Avian influenza is another disease of interest, especially in mute swans. In Europe, mute swans have been implicated in carrying H5N1. Serum samples were collected to evaluate exposure and combined cloacal/ oropharyngeal samples were collected to further identify the specific serotypes of avian influenza circulating in mute swans. Swan samples were also submitted for avian bornavirus testing, a disease previously thought to be found only in parrots and other pet birds.

Swans were lethally removed and samples were collected within 1 hour of death whenever possible. Serum samples were collected for influenza, NDV, and toxoplasmosis exposure. A fecal sample and the intestine were collected for parasite analysis. Cloacal swabs were collected for *Salmonella* testing and a combined cloacal and oropharyngeal sample were collected for avian influenza and NDV testing. The entire head was collected for avian bornavirus testing.

During Fiscal Year 2011, 461 mute swans were sampled. Samples were collected from Michigan (56%), New Jersey (24%), New York (3%), and Wisconsin (0.4%). The results that have been completed to date are summarized in Table 1. Sampling will continue in Fiscal Year 2012. An additional 200 samples will be collected to allow further identification of NDV, Eastern Equine Encephalitis, St. Louis Encephalitis, LaCrosse Virus and intestinal parasites. Once testing is complete, the results will be assimilated into a manuscript that will be submitted for publication.

Table 1: Number of mute swan samples screened, along with positive and negative results, for five pathogen groups.

Disease	Negative	Positive	Pending
Newcastle	87	113	260
Salmonella	455	3	0
Parasites	348	74	39
Avian Influenza	35	149	275
Toxoplasmosis	214	41	0

Cooperative Activities

Canine Parvovirus

Canine parvovirus is a pathogen that can infect most canids. The disease likely emerged in domestic dogs in Europe during the 1970s and rapidly spread throughout the world in domestic and wild species. Scientists at the NWRC revealed that canine parvovirus entered western United States coyote populations during 1978 and serological data suggested it was enzootic in coyotes by 1980.

Figure 1: Coyotes are the primary samples being collected for canine parvovirus screening.

Canine parvovirus typically causes disease by infecting bone marrow, lymph nodes, spleen, and intestines in young animals that no longer have protection from maternal antibodies. The most common clinical sign is pronounced hemorrhagic enteritis (bloody diarrhea). Canids that become infected as adults often have no symptoms, but can remain infectious for up to 6 weeks. Although transmission through direct contact with infected animals is important, indirect contact with infected environments likely plays a more important role in the transmission and maintenance in a population. Canine parvovirus is extremely stable in the environment and transmission can occur when a susceptible animal has contact with feces, infected soil, or fomites.

While canine parvovirus can be controlled through environmental decontamination and vaccination of domestic animals and wild canids in captivity, implementation of such protocols in wild populations is not currently practical.

Although viral shedding has been documented in wild canids, most studies to date have focused on serological surveillance. To gain a better understanding of canine parvovirus in wild coyotes, the NWDP is initiating a collaborative surveillance effort with Cornell University to determine the distribution of canine parvovirus by identifying viral particles in tissues and feces.

NWDP staff have developed a surveillance protocol that is currently being used by WS personnel to collect samples for canine parvovirus analysis. States that have access to coyote samples have been encouraged to work with their wildlife disease biologists to coordinate the sampling effort.

Cooperative Activities

Porcine Circovirus 2

Nearly all domestic swine herds are infected with Porcine Circovirus 2 (PCV2) worldwide. Although infection with the virus does not always cause disease, a suite of porcine circovirus associated syndromes and diseases (e.g., post-weaning multisystemic wasting syndrome) may occur in domestic swine when co-infections with PCV2 exist or various environmental (husbandry) or genetic (sow) risk factors are present. Vertical and horizontal transmission of PCV2 has been documented, and fecal/oral transmission has been identified as the most important route of infection within domestic swine facilities. Limited geographic sampling of feral swine in the United States has shown that PCV2 exposure in feral swine may mirror exposure levels seen in domestic swine herds, although many questions remain unanswered. In some areas, the relatively high apparent prevalence of PCV2 antibodies in feral swine is suggestive of efficient transmission of the virus within populations, or among feral and domestic populations.

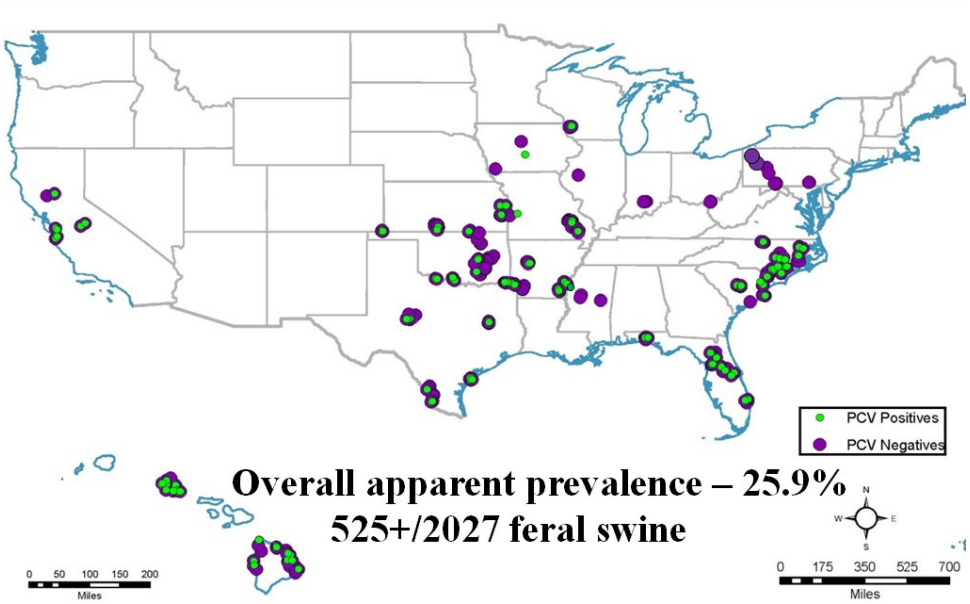


Figure 1: Porcine circovirus 2 sample distribution. Positive samples in green; negative samples are in purple.

The main objective was to determine the extent of PCV2 exposure in United States feral swine populations and its association with domestic swine production.

More than 2,495 samples from the NWDP's feral swine serum archive were sent to the Rollins Animal Disease Diagnostic Laboratory in North Carolina to be tested for presence of antibodies to PCV2. This retrospective survey covered feral swine populations from 72 counties in 18 states. Feral populations were chosen from counties representing a range of domestic swine production levels to help determine the relationship between domestic swine and the levels of PCV2 exposure seen in feral populations.

The results suggest that statewide PCV2 prevalence in feral swine may exceed 50% in some instances and that the majority of the states contain PCV2 infected feral swine. Initial analyses suggest that PCV2 exposure in feral swine is not correlated to the number of domestic swine and/or the number of facilities within a county of interest. This suggests that PCV2 may be independently maintained in feral swine populations. Future analyses to determine PCV2 genotypes (a or b) should provide more in insight into PCV2 virus movement between domestic swine operations and local feral swine populations.

Cooperative Activities

Porcine Reproductive and Respiratory Syndrome

Porcine reproductive and respiratory syndrome virus (PRRSV) was discovered in United States domestic swine populations in the mid 1980's. This virus may cause late term reproductive failure in pregnant sows or pneumonia in neonatal, nursery and even grow/finish stage pigs. The virus is widespread in the domestic swine industry worldwide and causes an estimated $560 million in annual losses in the United States alone. A 2006 study investigating PRRSV in 17 swine producing states found an average of 50% of grow-finish pigs to be positive within the 71% of swine production sites where the disease was found. Transmission occurs mainly by direct contact with infected individuals (or infected semen in breeding facilities) however, indirect and aerosol transmission have been shown to move the PRRSV between production facilities.

Figure 1: Feral swine

Now that endemic diseases such as pseudorabies (PRV) and swine brucellosis (SB) have been eliminated from the domestic swine industry, the focus of producers has shifted to the control and eventual elimination of PRRSV in the United States. A potential threat to the success of this eradication effort would be a reservoir of PRRSV circulating independently in feral swine populations.

Limited geographic sampling of feral swine in the United States has shown that PRRSV exposure in feral swine tends to be minimal and antibodies were found in fewer than 5% of individuals tested. These findings are in line with what has been documented in wild boar populations in other parts of the world. It has been suggested that feral swine may not be able to maintain PRRSV in their populations in the absence of disease spillover from domestic swine in the vicinity. PRRSV eradication efforts in the domestic industry may be compromised if the maintenance and potential reintroduction of PRRSV by feral swine is discounted. The main objective was to determine the extent of PRRSV exposure in United States feral swine populations to begin to evaluate their potential as a reservoir of the disease.

More than 2,495 samples from the NWDP's feral swine serum archive were sent to the Rollins Animal Disease Diagnostic Laboratory in North Carolina to be tested for presence of antibodies to PRRSV using the industry standard 2XR and X3 PRRSV enzyme linked immunosorbent assay's. This retrospective survey covered feral swine populations from 72 counties in 18 states. Feral populations were chosen from counties representing a range of domestic swine production levels to help determine the relationship between domestic swine and the levels of PRRSV exposure seen in feral populations. Enzyme linked immunosorbent assay test results documented fewer than 5% of all serum samples positive for PRRSV antibodies. The geographic distribution and state/county level apparent prevalence of PRRSV exposure in feral swine are currently being determined.

Nobuto Long-term Storage Study

The collection and transport of high quality samples is one of the primary challenges associated with wildlife disease surveillance. Samples must be able to retain useful information after being collected from field locations that are often remote, and under conditions that are less than ideal for maintaining biologically viability. Viable long-term storage of these hard to obtain samples is also a goal of many disease surveillance programs. A recent project has been initiated by the NWDP, in collaboration with the CDC to address the short- and long-term stability of plague antibodies on blood-soaked Nobuto strips under different environmental storage conditions.

The Nobuto strip is essentially a filter paper product that wildlife disease biologist and other field personnel use to collect blood samples for diagnostic analysis. A small amount of blood is collected on a Nobuto strip, which absorbs the sample and is allowed to air dry. The sample is then placed into a manila envelope. While this protocol has been used extensively in disease studies for many decades, a robust analysis that quantifies optimal storage conditions for blood-soaked Nobuto strips has yet to be completed.

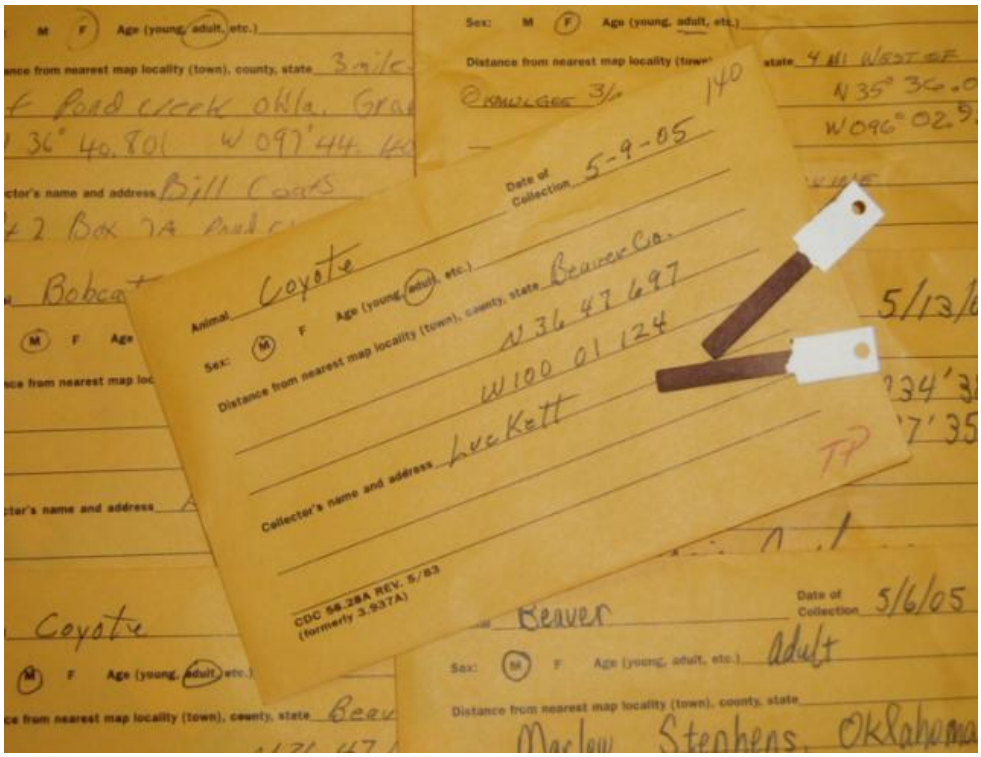


Figure 1: Blood-soaked Nobuto filter paper strips and data storage envelopes.

Data obtained through this study will provide robust information on the optimal storage conditions for Nobuto strip samples collected during the course of wildlife disease surveillance programs. Data will also help determine how best to maintain a long-term National Nobuto Archive, that will provide samples upon request from scientists.

Multiple Nobuto samples from known plague seropositive coyotes were collected and stored under ambient, 4^0 C, and - 20^0 C conditions. Humidity was monitored as well. Samples from all three temperature regimes were then tested periodically over time to determine if antibody titers changed over time. The samples are now being analyzed to determine the most effective environment to preserve sample integrity and to reduce protein degradation. Results are pending on this project.

Cooperative Activities

Japanese Encephalitis and Chikungunya Host Competence

The increasing movement of people, animals, and commodities around the world has the potential to translocate infectious diseases. The primary concern is not only the probability of foreign infectious diseases entering the United States, but that possibility that there are potential hosts that can become infected with, and go on to transmit, these new pathogens. A recent example of this occurred in 1999, when West Nile Virus invaded the United States and has since become established.

The NWDP and CSU are collaborating to determine whether wildlife and domestic animals in the United States can serve as viable reservoirs for two exotic viruses, Japanese encephalitis (JE) and chikungunya virus, that have the potential to spread to the country. In addition, this project seeks to assess the risk of introduction and establishment of these two pathogens into North America.

WS personnel baited and trapped wildlife using cannon nets, leg snares, Sherman traps, and Tomahawk® traps. Once captured, the animals were immobilized or physically restrained and loaded into animal carriers. The animals were then transported to the CSU Large Animal Biosafety Laboratory Level 3 (BSL-3) facility, where the studies were conducted.

Once inside the BSL-3 facility, the animals were inoculated with JE or chikungunya either through intramuscular injection or by using infected mosquitoes. Blood was drawn on specific days post inoculation and observational data was collected throughout the 21 day time-course of each study. Whole blood was centrifuged and the serum was analyzed by Vero cell plaque assay to determine viral titer. At the end of each study, animals were euthanized by intravenous injection of sodium barbital and organ tissues were then collected and examined for viral infiltration and tissue damage.

For JE, which is a Flavivirus, data showed that a variety of wild bird species are susceptible to infection. Interestingly, birds known to be amplifying hosts for West Nile virus, also a Flavivirus, are not susceptible to infection by JE virus. To date, no overt disease has been observed in any of the birds tested. Additional experiments are currently underway that are examining exotic reptile and mammal species in the United States. Data suggest that many can become infected and possibly amplify JE virus without apparent symptoms.

Chikungunya virus, on the other hand, has not been shown to infect any of the eleven bird or mammal species tested to date. We plan to continue evaluating wildlife for susceptibility to both viruses. Vector studies have already implicated several *Culex* and *Aedes* mosquito species that are prolific in the United States to be competent vectors for both viruses. Manuscripts reporting the results of these data have been submitted for publication.

The next step in the project is to start a baseline sero-surveillance project by collecting serum samples from birds, mammals and reptiles collected in Guam, Hawaii, and nearby islands. The serum samples will be tested for neutralizing antibodies to JE and chikungunya viruses. If positives are identified, additional sample collection and testing may occur.

Cooperative Activities

Assay Development for Tularemia Surveillance

Tularemia is a bacterial disease caused by *Francisella tularensis*. The organism infects and induces disease in a broad range of mammalian hosts, including humans. Each year, hundreds of infections are documented in people in the United States and the disease is considered, in some forms, to be the most infectious bacterial agent ever documented.

Figure 1: *Francisella tularensis* bacterial colonies.

The Colorado School of Mines, Department of Chemistry, Advanced Biodetection Laboratory, in cooperation with the NWDP, have been developing a field ready immuno-chromatograghy detection system for tularemia. The system utilizes small organisms called bacteriophages, or simply phages, which are essentially viruses that infect bacteria. The strategy behind the technology is that a serum sample would be collected from an animal, mixed with a phage media, and then placed in a small plastic case with immuno-chromatography paper. If tularemia is present in the blood sample, the phage would infect the bacteria and multiply much like viruses or bacteria when they infect a host organism. When the phages multiply, they will migrate up the immuno-chromatography paper and show a colorimetric positive, supplying the biologists with results in minutes or hours rather than days or weeks. If the bacterium is not present in the sample, the chromatography paper will remain colorless or blank.

The main focus of this project has been to capture native deer flies and ticks for characterization of their commensal microbial flora, with the primary goal of assessing these insect species as potential natural reservoirs of *Francisella tularensis* – specific bacteriophages.

Tick collection has typically been carried out by drag flag sweeping. Ticks have also been opportunistically collected off of feral swine or other mammal species during WS operational activities. Deer flies are collected using the trolling trap method. To date, 10 state WS programs have participated, or shown interest in participating, in this project.

Current results have not yet indicated the presence of any *Francisella* phages in ticks or deer flies; however, some samples have demonstrated lytic activity against *Francisella tularensis* in bacterial plating experiments. Data collection is ongoing.

Cooperative Activities

Comparison of Influenza Viruses in Wildlife and Humans

The NWDP is collaborating with ASU, The Mayo Clinic, and CSU to determine relatedness of influenza A viruses circulating in wildlife and humans in the Southwestern United States. The project, supported in part by a 2011 ASU-Mayo Clinic Seed Grant, is exploring the potential of using the health of wild animals as sentinels for zoonotic diseases in humans.

An example from previous research of how the relatedness of viruses can be viewed with the ZooPhy tool created by Dr. Scotch,.

This study uses samples collected during wild bird surveillance activities and stored in the Wild Bird Tissue Archive which was established in 2006. Genetic material from influenza A matrix positive samples are being harvested by CSU personnel using virus isolation and lysis techniques. Once harvested, extracted RNA is sent to ASU where the hemagglutinin (HA) segment of the genome is amplified. Cloning is then conducted using plasmid vectors transformed into competent E-coli cells. The plasmids are incubated overnight, extracted, and sequenced. The sequences are then assembled and the influenza subtype is determined by using the National Center for Biotechnology Information Basic Local Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi). The avian sequences will be compared with the HA sequences of human cases using a variety of bioinformatics techniques. One approach is to use Dr. Scotch's bioinformatics tool *ZooPhy* (zoophy.wikispaces.asu.edu), which models the dispersion of influenza in avian species and humans across the Southwest United States.

Thus far, 228 matrix positive samples have been harvested. Of those samples, seven viable viruses have been identified and genetic material sent to Arizona for genetic sequencing. Currently, the seven HA sequences have been completed and four of these have been published in GenBank with the following accession numbers: JN673246, JN864063, JN798212, and JN590044. The avian subtypes are: H8N4 (1), H4N6 (2), H11N9 (1), H3N8 (1), H6N1 (1). One sample is currently being sequenced and preliminary results suggest it is H3N8.

Cooperative Activities

Immunological Response of Coyotes to Plague Infection

The NWDP coordinates a long-term plague surveillance project that opportunistically collects blood samples from coyotes that are removed during wildlife damage management operations. Serology results that detect plague antibodies in these blood samples can indicate if coyotes have been exposed to plague, which provides information on plague distribution and activity in wildlife, and the associated risks to people and domestic animals. A limitation with this approach is the lack of knowledge regarding coyote immunological responses to plague exposure. Specifically, there has historically been limited information on the strength of the antibody response, the duration that antibodies can be detected after exposure or re-exposure, and how the antibody response decays over time. The goals of this project are to characterize antibody responses in coyotes exposed to *Yersinia pestis*, through two different transmission routes.

Figure 1: Coyote

The NWDP collaborated with CSU, CDC, and the NWRC to assess plague infection and antibody response in coyotes. Coyotes were obtained from the NWRC Predator Research facility and were housed at the CSU Large Animal Disease Laboratory, Biosafety Level 3 facility in Fort Collins, Colorado. One experimental coyote cohort was inoculated intradermally to simulate transmission via flea bites. Another cohort was inoculated orally by allowing coyotes to feed on a *Y. pestis* infected mouse carcass. Blood was collected from individual coyotes before and at specific time intervals after the initial inoculation to document the antibody response. Three months after the initial inoculation, a second inoculation was administered to determine if the coyotes would produce an anamnestic response.

Data analysis is ongoing, but information obtained during the course of this study has already provided unique insight into the immune responses of coyotes via different routes of exposure. These efforts will hopefully allow researchers conducting plague surveillance in coyotes to pinpoint more precisely the timeframe of recent plague activity.

Cooperative Activities

Detecting Infected Animals

Human health is inexorably linked with the health of our ecosystems and the animals that occupy them. Consequently, the USDA/APHIS monitors the health and disease state of wild and domestic animal populations in the United States and abroad. In support of this mission, the NWDP has been collaborating with NWRC, Monell Chemical Senses Center, and CSU in developing novel approaches to detect and remove animals in the prodromal period of a disease state as a potential tool in managing disease spread.

Figure 1: Experiments indicate that mice can identify odors associated with avian influenza.

Healthy animals have unique "odor types" or patterns of volatile chemical signatures emanating from their external processes, e.g., skin, breath, urine, and feces. When a virus or bacteria enters an animal's system and infects the animal, processes to fight off the infectious agent are engaged. Metabolic changes accompany these physiological processes, resulting in alterations of the odor. Development of a technique that could detect such odors could lead to a valuable tool in rapid detection of diseases.

The objective of this study is to develop techniques to detect odor changes of duck feces resulting from infection with a low pathogenic avian influenza virus. The first stage of this project is to train live mice biosensors to detect odors associated with infection. If mice prove to be effective, a canine model can similarly be developed for field deployment. The second stage of the project is to develop an instrumental technique to quantify odor changes and potentially lead toward mechanical sensors for disease surveillance.

Mallard ducks were infected with a low pathogenic avian influenza at the CSU Animal Disease Laboratory. A daily pre-inoculation fecal sample was collected from each individual and pooled. Daily post-inoculation fecal samples were collected from each individual starting at day 2 post-infection. Samples were homogenized, irradiated, and shipped to NWRC at the Monell Chemical Senses Center on the campus of the University of Pennsylvania.

When paired with uninfected feces, the mice biosensors identified the infected feces 90% of the time. When novel sources of feces were presented, the biosensors correctly identified infected samples with greater than 70% accuracy.

The instrumental analysis using data from gas chromatography/mass spectrometry system was subjected to a principle component analysis. The results yielded a model for assessing infection status on the basis of fecal volatiles. Further research is ongoing to determine the importance of sample irradiation for generating the relevant metabolites.

Chronic Wasting Disease

Figure 1: Elk at fence line.

Chronic wasting disease (CWD) was discovered in Colorado and Wyoming in the early 1960's. CWD is a chronic, progressive, fatal, neurologic disease affecting mule deer, white-tailed deer, elk and moose. In the 1990's, CWD was discovered in captive cervid herds. Until about 2000, the disease in wild animals was believed to be limited mostly to Colorado and Wyoming with limited incursion into surrounding states. In 2002, the disease was discovered in southern Wisconsin, far from its original focus. The finding of this disease in the Midwest initiated funding for intense surveillance throughout most of the United States. Intensive surveillance continued for about the next decade.

CWD in free-ranging cervids has been detected in 16 states, mostly surrounding the original endemic areas of Colorado and Wyoming as well as southern Wisconsin and northern Illinois. CWD was detected in West Virginia in 2005 and a few positive cases have been detected in the surrounding states of Maryland and Virginia. In Minnesota and New York, a few positive animals have been found in close proximity to depopulated infected captive farms. The evidence suggests that CWD will continue to slowly spread throughout the North America. Despite intensive research efforts, there is currently no effective control strategy at this time.

The NWDP provides assistance for CWD surveillance throughout the United States. In addition, the program has assisted with developing assays that can better detect infected animals, worked closely with the WS operational program with depopulation of infected cervid farms, provides subject matter expertise on CWD, and is actively involved in collaborative CWD research with CSU, ARS, VS, and the Canadian Food Inspection Service.

One of the main problems in dealing with the transmissible spongiform encephalopathies, such as CWD, scrapie, and mad cow disease, is the lack of a live-animal test that would allow for early detection of the disease. Researchers in Norway and the United Kingdom discovered that there was a band of relatively accessible rectal lymphoid tissue that could be biopsied in live sheep. In order to generate the number of samples needed to validate the rectal biopsy procedure for diagnosis of CWD in white- tailed deer, the NWDP, VS, Canadian Food Inspection Agency, and CSU are collaborating to examine infected herds in Canada and the United States.

Bovine Tuberculosis

Figure 1: Deer and calf interacting near feed.

Bovine tuberculosis (TB) is a chronic bacterial disease (primarily of cattle) caused by the microorganism *Mycobacterium bovis*. The disease can also infect other species, including humans and wildlife. Bovine TB is most often transmitted to humans by inhalation of aerosolized respiratory tract bacteria, ingestion of unpasteurized milk, and inoculation by contaminated instruments (such as knives). The disease can be spread from livestock to wildlife or wildlife to livestock via the fecal-oral route, ingestion of contaminated food, or by respiratory tract secretions. The APHIS Bovine TB Eradication Program and state departments of agriculture have dramatically reduced TB in the United States cattle herd; however, TB is still present in some cattle, and spillover into wildlife and their potential to serve as a reservoir is a concern.

The first documented cases of bovine TB in North American wildlife included white-tailed deer in 1933, 1937, and 1961. All of those cases were in the same area of New York. TB was subsequently found in free-ranging white-tailed deer in northern Michigan in 1975. A second occurrence from the same area of Michigan in 1994 drew attention to the possibility of wildlife being a reservoir host for *M. bovis*. Since 1995, *M. bovis* has been detected in several wildlife and feral species in North America, including white-tailed deer, mule deer, elk, bison, moose, raccoons, coyotes, opossums, feral cats, grey fox, black bears, feral swine, gray wolves, red fox, and bobcat, with varying degrees of infection.

The NWDP provides support for wildlife testing in states that have discovered *M. bovis* in livestock. Numerous wildlife disease biologists have been deployed to provide assistance in wildlife TB testing in Minnesota and Michigan, where spillover into white-tailed deer has occurred.

In collaboration with VS, the NWDP has developed English and Spanish versions of the "Guidelines for Surveillance of Bovine Tuberculosis in Wildlife," which is available in bound copy, as well as electronically. The program introduced these guidelines to the United States/Mexico Bi-national Tuberculosis Committee at the 2012 winter meeting in Nashville, Tennessee.

In 2011, the NWDP accepted an invitation by the Mexican Ministry of Agriculture (SAGARPA) to lecture and provide laboratory demonstrations on tuberculosis in wildlife. At the request of Mexico, the NWDP has agreed to provide, training in hunter surveillance sampling for TB during the 2012 deer hunting season.

International Wildlife Disease Collaborations

The transmission of, and infection by, wildlife diseases are a global concern. Wildlife species are active in almost every environment, and often there is the potential for interactions with domestic animals. Seasonal migrations and natural species dispersals can spread pathogens and disease significant distances; migrations can cover thousands of kilometers, crossing many countries and covering multiple continents. These movements are generally not well understood and impossible to control. Whether these species serve as potential bridge species to transmit diseases or as hosts to enable pathogens to persist is often unknown, and potential problems are frequently not investigated. International collaborations increase our understanding of potential threats and knowledge of emerging zoonotic diseases. While sharing disease monitoring networks enables neighboring countries to be better prepared and coordinate control tactics, collaborative workshops and scientific exchanges provide valuable experiences for our scientists to work with foreign diseases before they enter the United States. We have contributed to improving the capacity to conduct surveillance for wildlife diseases in partner countries. Working alongside foreign biologists not only facilitates exchange of knowledge that is mutually beneficial, but also increases the trust and familiarity among agencies that is essential when preparing for, or responding to, an emerging issue.

Figure 1: Live bird markets in Indonesia.

NWDP has several long term activities addressing wildlife disease issues. Over the past several years, the program has conducted wildlife disease surveillance or training for researchers from over 30 countries spread across Asia, Africa, and South America. The NWDP serves as a national associate on the Food and Agriculture Organization of the United Nation's Scientific Task Force on Wildlife Diseases, and provides technical expertise for USFWS – United States & People's Republic of China Nature Conservation Protocol Joint Committee Meeting. The NWDP collaborated with Chinese Academy of Sciences and the Chinese State Forestry Administration to conduct the Asian Pacific Conference on Wildlife Borne Diseases. Subsequently, staff collaborated with Chinese Academy of Sciences, Institute of Zoology and Harbin University to develop and conduct training for Chinese State Forestry Administration on wildlife disease surveillance. In addition NWDP supported developing additional workshops in Peru to train specialists in conducting wildlife disease surveillance. The NWDP also responded to a request to lecture on TB in wildlife and to teach multiple wet labs on necropsy techniques in cervids in Mexico City. There is also an on-going collaboration with the Danish government to conduct avian influenza surveillance in wild birds in Greenland, along with a collaboration with the Mongolian Academy of Science to expand surveillance of avian influenza across the breeding grounds of the bar-headed goose, as well as 13 other species, in Mongolia.

Collaborative projects with CSU are being developed to identify diseases in bats and to identify potential wildlife reservoirs for Chikungunya viruses in Cambodia. Collaborative efforts with CSU are also underway to finalize graduate student projects on disease transmission in wildlife markets in Indonesia.

International Wildlife Disease Collaborations, continued

The NWDP has also begun collaborating with multiple partners to assist with investigating the role of wild Suidae in the potential spread of African swine fever to domestic swine. Two activities are addressing European Wild Boar (*Sus scrofa*) in the Ukraine. The NWDP is collaborating with USDA, Foreign Agricultural Service to improve capacity within Ukraine to conduct disease surveillance in wildlife. Specific objectives for this project include strengthening Ukraine's wildlife management and disease surveillance program, promoting effective and efficient use of samples collected from the field to broaden surveillance of animal diseases, and strengthening existing response to emerging disease outbreaks in wildlife. The NWDP also hopes to gain first hand experience in surveillance and control of ASF. Another related collaborative project with the Southern Research Institute is addressing capacity in Ukraine to collect and process samples from wild boars for African swine fever and Classical swine fever. The NWDP also collaborated with multiple partners in Uganda and Kenya to improve understanding of African swine fever in native wildlife species. Collaborations with Makerere University and Swedish Agricultural University will apply a molecular ecological approach to understand the role of bushpigs in the epidemiology of African swine fever virus at the wildlife-livestock interface in Uganda. A similar collaboration with US Army Medical Research Unit-Kenya, Central Veterinary Laboratories Kabete, the International Livestock Research Institute, and others, is investigating African swine fever in desert warthog wild pig populations in Northeastern Kenya.

Figure 2: Avian influenza sampling with collaborators in China.

NWDP played a primary role in implementing a Memorandum of Understanding between the Chinese Academy of Sciences, Bureau of Life Sciences and Biotechnology, and Wildlife Services to promote ongoing cooperation. It targets several areas for desired collaboration on wildlife disease issues, including identifying harms and risks posed by wildlife disease that threaten agriculture or have zoonotic implications; developing a joint Consortium to address emerging disease issues in wildlife populations; identifying risks and developing management approaches to mitigate zoonotic disease impacts; promoting global awareness of the threats posed by emerging wildlife diseases; and developing activities to enhance the region's capacity for emerging wildlife disease response. It also pinpoints activities related to protecting agriculture and natural resources from wildlife species, identifying potential issues posed by wildlife on or near airports and developing methods and management approaches to mitigate wildlife hazards to aviation activities; and promoting development of methods and management approaches to reduce wildlife disease risks to human health and safety. A NWRC Program manager serves as the co-chair for the bilateral working group formed to provide guidance on joint activities. Thus far, the bilateral working group has agreed to host biannual regional conferences on wildlife diseases in Asia and to collaborate on surveillance activities for wildlife borne diseases. The plan is to develop an Asia-Pacific Wildlife Disease Network and to provide training for the Chinese State Forestry Administration to conduct wildlife disease activities.

International Cooperation

Greenland and Denmark

Cooperative projects in other countries enable the NWDP to extend the range of surveillance in support of the Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds. With the appearance of highly pathogenic avian influenza (HPAI) H5N1 in wild birds in western Europe during fall of 2005, the risk of a wild bird introduction of HPAI H5N1 to North America via a migratory "bridge" in Greenland increased. By March of 2006, HPAI H5N1 had been found in wild birds in Italy, Germany, Switzerland, Austria, Poland, France, Sweden and Denmark.

Figure 1: Avian influenza sampling in Greenland.

Greenland lies in an overlap zone between western European flyways and the North American Atlantic flyway. Greenland White-fronted Geese come from wintering grounds in Europe to breed in Greenland in close proximity to Canada geese that winter in the northeastern United States. Both species of geese have opportunities to comingle with resident and migratory mallards in southern Greenland. All three species are competent hosts of avian influenza. Greenland is therefore considered a potential route for transfer of HPAI H5N1from Europe to North America.

The NWDP has had cooperative agreements with counterparts in Denmark since 2007 to conduct wild bird surveillance for avian influenza in Greenland. Jointly financed by the NWDP and the Danish Veterinary and Food Administration, the surveillance project has been carried out in collaboration with the Technical University of Denmark's National Veterinary Institute, Aarhus University's National Environmental Research Institute, and the Greenland Institute of Natural Resources.

Most of the samples have been collected by Danish and Greenlandic biologists and game managers. WS biologists conducted independent sampling in remote northwestern Greenland in 2008, and participated with an international team in sample collection in 2009. The project has resulted in collection and diagnostic testing of 4,086 samples from 24 species of birds between 2007-2010. There have been no H5/H7 samples found and only six samples have been matrix positive for non-H5/H7 avian influenza. These results suggest that while Greenland is a possible route of HPAI entry to North America, the risk may be quite low. In Fiscal Year 2011, the target was an additional 1,200 samples from mallards and Icelandic gulls, which are the two species diagnosed as carriers of low pathogenic avian influenza in previous years. Actual sample numbers and results are pending. As in the United States, the future plan is to curtail active surveillance in Greenland.

International Cooperation

Indonesia

Highly pathogenic avian influenza (HPAI) H5N1 has established itself as an endemic disease in Indonesia, and mortalities in humans and domestic poultry from HPAI infection remain relatively high levels in the country. Wildlife are believed to play a key role in the epidemiology of this disease, but the specifics of their involvement has yet to be defined. Additionally, some cultural practices (i.e., wild animal markets) that have been ongoing for generations may contribute to the persistence of the virus in Indonesia.

The NWDP, in collaboration with CSU) has ongoing projects on HPAI in Indonesia (West Java). This work has established a research veterinary diagnostic laboratory at Bogor Agricultural University. The laboratory is currently in the process of assaying approximately 8,000 HPAI diagnostic samples that have been collected from the region. Earlier this year, the Indonesian-United States collaborative team completed a cross-sectional study on the prevalence of avian influenza in domestic ducks, and is now in the process of conducting a longitudinal study that will focus not only on domestic ducks, but will expand to include wild birds and rodents as well.

The work in Indonesia currently focuses on domestic poultry, human-poultry interactions, and free-ranging wild birds and mammals. One aspect that has come to our attention in Indonesia is the role of live, wild animal markets in the daily lives of Indonesians. These markets are supplied with wildlife (i.e., birds, mammals, and reptiles) that have been captured from the surrounding regions. Animals are comingled in very close proximity with little separation, which creates opportunities for viral transfer between species.

Figure 1: Training sessions and presentations in Indonesia.

Indonesia, continued

Animals are purchased live and kept as pets or slaughtered for human consumption, which creates a unique human/wildlife interface that could perpetuate the HPAI H5N1virus and pose another route of exposure for human infection.

The NWDP, in collaboration with CSU and Bogor Agricultural University, are conducting wild animal market surveillance and testing in conjunction with ongoing investigations of HPAI in Indonesia. Objectives of this work are to determine the origin of wild animals in markets, if intermediate animal collection points occur, and document the distribution pathways and endpoints of wild animals in markets. This work will also tests samples for HPAI H5N1 from captive wild birds and mammals in these markets. Samples were also collected from drinking water provided to the animals.

Also, NWDP and CSU researchers met with officials at Bogor Agricultural University to set project goals and develop a basic course of study for graduate students on the project. Training on the safe capture and handling of wild birds and proper techniques for bleeding small birds was also provided.

Figure 2: Sampling domestic poultry in Indonesia.

In cooperation with the Indonesian Veterinary Organization, Center for Indonesian Veterinary Analytical Services, the team traveled to the Tangerang District northwest of Jakarta where field studies of wild birds and rodents were being conducted at two different domestic bird production facilities. The Tangerang District has been one of the more active districts for HPAI outbreaks in village-raised poultry and waterfowl. The team documented possible interactions between wild birds and rodents with the domestic ducks at each facility. Samples from the wild birds and rodents are being analyzed for the presence of HPAI virus and antibodies. The data obtained from these projects will be valuable in determining if wild birds and rodents have a role in maintaining HPAI in Indonesia.

International Cooperation

Mongolia

The NWDP has been collaborating with colleagues in the Mongolia Academy of Sciences and CSU for three years on a monitoring project for highly pathogenic avian influenza (HPAI) in Mongolian wild birds. Samples have been collected in Mongolia where a number of migratory bird die-offs have occurred due to the virus.

Figure 1: Sampling swans at Khar Us Lake, western Mongolia (photo by Nyambayar Batbayar).

The bar-headed goose was the principle species involved in the well-known HPAI H5N1 wild bird outbreak at Qinghai Lake in North-central China in 2005, as well as a die-off at Erkhel Lake in Mongolia in 2006. Both sites are in the Central Asian flyway. The Qinghai Lake event was the first documented large scale die-off of wild birds due to HPAI H5N1. Thousands of bar-headed geese died at Qinghai Lake, about 10% of the entire population, which is now estimated at about 36,000 birds.

Mongolia presents unique advantages for the study of avian influenza in wild birds. About 70% of the bar-headed goose population breed on Mongolia's vast grasslands and wetlands each year. The natural ecology of avian influenza in wild birds can be studied in isolation from interactions with domestic fowl because there are almost no commercial domestic poultry or backyard poultry in Mongolia. In addition, large numbers of other waterfowl and shorebirds breed or pass through Mongolia during annual seasonal migrations along the Central Asian flyway. In the Palearctic zone, the East Asian-Australian flyway bridges the gap between the Central Asian flyway and the North American Pacific flyway. Thus, through flyway interactions, there is potential for HPAI H5N1 to cross into the North American Pacific flyway via wild bird movements.

Since 2009 biologists with the Wildlife Science Conservation Center and the Institute of Biology at the Mongolian Academy of Sciences, have collected approximately 2,000 samples from birds and small mammals, including tracheal swabs, fecal samples, serum, and tissues. The samples are packed in nitrogen vapor shippers and shipped from Mongolia to CSU, where they are tested for influenza virus. Samples are assayed by real-time reverse-transcription polymerase chain reaction in a Biosafety Level 3 laboratory at CSU. Positive samples are further characterized by virus isolation methods and genetic sequencing.

In Fiscal Year 2011, 549 swab samples from 16 species were collected in Mongolia and transported to CSU for avian influenza testing. Diagnostic results are pending.

International Cooperation

Canada and Ecuador

Surveillance for avian influenza in wild birds in Canada is of interest to the United States for several reasons. First, Canada has recently had two outbreaks of HPAI H7N3 in domestic poultry; in British Columbia in 2004 and in southern Saskatchewan in 2007. Both outbreaks are thought to have originated with the introduction of a low pathogenic form carried by wild birds, spreading to domestic poultry and mutating into a highly pathogenic form. Second, the 'prairie pothole' region in southern Saskatchewan and Alberta are part of huge complex of wetlands spanning three Canadian provinces and five states in the United States, where multitudes of migratory waterfowl, the principle natural reservoirs of avian influenza, comingle each fall and spring. This creates a situation amenable to mixing and re-assortment of avian influenza subtypes. Third, Canadian surveillance serves as an early warning system for entry of influenza viruses into the 48 contiguous United States.

Canada has conducted surveillance for HPAI H5N1 in wild birds since 2005. The interagency initiative includes federal and provincial government agencies and several universities. The two components of the program are seasonal surveillance of live waterfowl species from selected regions, and survey of avian mortality events. Unlike the United States, Canada does not sample hunter-harvested birds for avian influenza. One consequence of this is a much smaller annual sample size. NWDP has joined into annual cooperative agreements with the University of Saskatchewan since 2007 and continuing into 2011, with the objective of boosting surveillance for HPAI H5N1 in the prairie pothole regions of Saskatchewan and Manitoba.

In addition, this collaboration supports a related project on capacity building for HPAI surveillance in Ecuador. Blue-winged teal breed in Canada and the United States each summer, then migrates to Mexico, Central America and northern South America each winter. Canada has bird band recovery data for tens of thousands of blue-winged teal dating back to the 1920's. This makes the blue-winged teal a model species for study of the possible distribution and spread of avian influenza through the Americas. Meanwhile, with United States and Canadian assistance, Ecuador has been able to acquire laboratory diagnostic equipment to perform real-time reverse-transcription polymerase chain reaction screening of samples. A laboratory technician from Ecuador has been trained in Canada to perform the testing. The University of Saskatchewan has also worked to develop a network of Ecuadoran academic, government, and poultry industry representatives to coordinating and conduct systematic surveillance of wild birds. Ecuador can now monitor blue-winged teal and other wild birds for avian influenza, thereby extending the reach of the United States HPAI H5N1 early detection program into South America.

Figure 1: Sampling blue-winged teal in Canada for avian influenza.

Emergency Management

Response to Emergencies

The key to effective emergency response is a strong network of people trained and prepared to respond when the need arises. Responding to emergencies is common for WS. On most days every state receives multiple calls to assist with a problem or resolve an issue. WS has a cadre of professionals for whom these emergencies are routine, though each often shows its own unique characteristics. Most responses occur at the local or state level. However, occasionally additional resources are required for larger emergencies of to meet specialized needs for skills or equipment. The NWDP coordinates national emergency response capacity within WS. Program staff works with ESF-11 Coordinators to maintain an awareness of available resources through WS and ensure that the procedures and protocols required for initiating and implementing a response are available. The NWDP also ensures a cadre of employees is trained and prepared to respond to disease outbreaks in wildlife that threaten agriculture or human health.

During Fiscal Year 2011, NWDP staff contributed to APHIS response capacity by providing comments on drafts for the Emergency Qualifications Systems Guide and the ROSS Directory. Staff also coordinated with other APHIS Emergency Programs to develop and implement ESF-11 protocols, and served as liaison with ESF Emergency Response Regional Coordinators. NWDP participated on the WS Contaminants Response Working Group (CRWG) to ensure communication among WS employees developing specialized criteria for responses to emergencies involving contaminants (e.g., oil spills) and WS employees preparing for other emergency activities. NWDP and the WS CRWG worked together to develop and implement an EMLC Bioline project to enable Hazwoper Certification for approximately 100 targeted WS employees. A NWDP employee participated in assessing feasibility of a virtual course to fulfill ICS 300 Training. The virtual course would eliminate travel costs previously incurred while participating in training. The NWDP staff also reviewed WS Emergency Communications Standard Operating Procedures and other WS emergency related documents.

Figure 1: Emergency response trailer.

All wildlife disease biologists within NWDP are regarded as WS initial responders for any APHIS Emergency Response, and as primary responders for disease outbreaks in wildlife. These biologists are expected to maintain awareness of emerging wildlife disease issues, merge existing knowledge with new technical advances, and adapt to changing surveillance activities. Wildlife Disease Biologists also are expected to be alert for emerging wildlife disease issues in their assigned state. Recognizing concerns and being prepared to respond often requires an awareness of activities in other states with similar wildlife species or habitats that are prone to similar pathogen exposure. NWDP applies multiple approaches to assist Wildlife Disease Biologists to maintain necessary skills, abilities, and knowledge to perform well in these positions. Necropsy and foreign animal disease information is taught through formal courses. During Fiscal Year 2011, NWDP maintained first responder readiness of 42 Wildlife Disease Biologists by ensuring compliance with FOH medical and respirator fit-test requirements, and by providing surveillance and emergency response training at the

Emergency Management

Response to Emergencies, continued

NWDP Emergency Response and Wildlife Disease Surveillance Training Meeting in Pingree Park, CO. Wildlife disease biologists shared experiences and knowledge acquired on projects within their own states to enhance the program's capacity to address these issues when they emerge elsewhere. Topics include improving sampling and capture methodology, detecting wildlife diseases, and working with cooperators. Poster sessions provide more opportunity to exchange ideas. WS veterinarians conducted a 4- hour refresher course on immobilization and euthanasia drugs. This session coupled with other presentations and a field practicum on drug delivery systems contributed to fulfilling continuing education credits necessary to maintain employees' immobilization and euthanasia certifications.

Figure 2: Wildlife Services sharpshooters mobilized for Chronic Wasting Disease response.

NWDP maintains three mobile emergency response laboratories. These mobile units consist of a diesel pickup truck, laboratory trailer, and all-terrain vehicle. The laboratory trailer also comes equipped with a mounted generator, free standing generator, refrigerator, heater and air-conditioning unit, centrifuge, dissecting scope, optical microscope, autoclave, large animal walk -on scale, necropsy instruments, and various supplies and equipment for a variety of tissue collection procedures. These units can serve as clean rooms, necropsy labs, mobile command centers, and hunter-check stations.

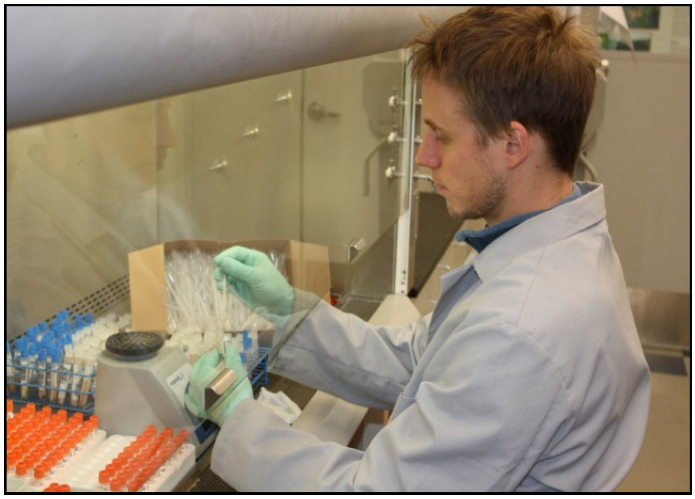
NWDP also provides coverage of an Emergency Hotline number (877-303-6363) to ensure rapid response to emergency requests. Calls to this number are forwarded to the assigned NWDP emergency contact person enabling response capacity 24 hours a day, every day.

NWDP successfully coordinated responses to 6 requests to address wildlife disease issues.

- Minnesota Chronic Wasting Disease surveillance; consisted of mobilizing 9 wildlife disease biologists during six mobilizations.
- Minnesota bovine tuberculosis surveillance; consisted of mobilizing 9 wildlife disease biologists during two mobilizations.
- Missouri Chronic Wasting Disease surveillance; consisted of mobilizing 2 wildlife disease biologists during a single mobilization.
- Nebraska Fall Hunter Harvest bovine tuberculosis surveillance; consisted of mobilizing 5 wildlife disease biologists during two mobilizations.
- Wisconsin Chronic Wasting Disease surveillance; consisted of mobilizing 3 wildlife disease biologists during a single mobilization.

Archives

Sample Storage



Figures 1 and 2: Wildlife samples are held in multiple archives for long-term storage.

The NWDP was established in 2003 with a mission to provide a nationally coordinated system of surveillance and emergency response to diseases of concern in wildlife. In support of that mission, the wildlife tissue archives were established in 2006. The diverse contents of the archives are derived from the Interagency Highly Pathogenic Avian Influenza Early Detection System for Wild Birds, routine surveillance for diseases carried by feral swine, as well as monitoring for plague and tularemia in wildlife. The collection is unique in the quantity of samples, the diversity of species, the broad geographic range, and consistent sampling effort over extended periods of time.

The NWDP Wildlife Tissue Archives consists of three collections: The Avian Tissue Archive is cooperatively administered with CSU's Veterinary Diagnostic Laboratory, which houses the collection on their campus in Fort Collins. The archive now consists of approximately 283,000 wild bird swab samples collected for highly pathogenic avian influenza surveillance in the United States from 2006-2011. Swab samples from 25,131 wild birds were collected in the first six months of Fiscal Year 2011. Samples represent 260 wild bird species from all 50 states and several US territories. Over 800 samples were loaned to six universities in Fiscal Year 2011 for retrospective disease studies.

The Feral Swine Serum Archive consists of serum samples from feral swine collected in 36 states. In Fiscal Year 2011, samples from 3,355 feral swine were added to the archive. The samples are used to monitor over a dozen diseases carried by feral swine.

The Plague and Tularemia Archive consists of over 13,000 whole blood Nobuto filter strips from 92 species of carnivores, rodents and other mammals, and several bird species. The most frequently sampled species are coyotes, beaver, raccoons, skunks and feral swine. More than 5,000 samples from 47 states were added to the archive in Fiscal Year 2011.

The NWDP considers sample requests for research projects, retrospective disease surveillance, or diagnostic assay development, on an individual basis.

Procedural

Surveillance Manuals

USDA/APHIS/Wildlife Services Procedures Manual for Canine Parvovirus (CPV) Surveillance

December 2011

Wildlife Services' Comprehensive Feral Swine Disease Surveillance Procedures Manual

Feral Swine Procedures Manual October 2010

October 2010

Hepatitis E Virus (HEV)
Procedures Manual

April 2011

USDA/APHIS/Wildlife Services
Procedures Manual for
Baylisascaris species
Surveillance

June 2011

Bluetongue (BTV) and Epizootic Hemorrhagic Disease (EHD)
Procedures Manual

2012

Trichinella and *Toxoplasma* Identification Project:
Genotyping *Trichinella* and *Toxoplasma*

October 2010

Plague and Tularemia Procedures Manual

Wildlife Services' National Wildlife Disease Program

April 2011

Protocol for Mute Swan Diseases Project

Objective
Samples will be collected from mute swans that are removed as part of the Great Lakes initiative in Wisconsin, Michigan, and New York. Additionally, samples will be opportunistically collected from swans in New Jersey, Rhode Island, Massachusetts, and Washington for wildlife damage management purposes. The objective will be to selectively test mute swans from each state (approximately 600 total) and submit them for *Salmonella*, Newcastle, *Toxoplasma gondii* and intestinal parasites. The results will be compiled into a manuscript with the goal of producing literature with relevance to swan health.

Collecting Samples
Salmonella
Use the provided Cary-Blair media/swab kit. The kit does not require special storage. However, it should be kept out of extreme temperatures. Open the packet, twist the top from the sterile media vial along the dotted line. Use the swab to collect a fecal sample and the return the swab to the media. Label the outside of the sample with one of the swan barcodes.

Newcastle Disease Virus
Collect 3 mL of blood from each swan (if possible). Centrifuge the blood and transfer at least 1 mL of serum to a 2 mL cryovial. If additional serum is available it can be stored in other cryovials and sent to the archive. Label each serum vial with the provided barcodes.

Swine Influenza Virus (SIV)
Procedures Manual

October 2010

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